PIPERETTINE FROM *PIPER NIGRUM*; ITS ISOLATION, IDENTIFICATION AND SYNTHESIS.

and

THE CONSTITUENTS OF THE NON-SAPONIFIABLE FRACTION OF *SPARTIUM JUNCEUM* L.

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by

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SUMMARY.

PART I. PIPERETTINE FROM PIPER NIGRUM; ITS ISOLATION, IDENTIFICATION AND SYNTHESIS.

The concentrated mother liquors from an ethanolic extract of Piper nigrum, after removal of successive crops of piperine, furnished a yellow-brown amorphous powder m.p. 100 - 112° from which piperettine, $C_{19}H_{21}O_3N$, a yellow-green crystalline solid m.p. 146 - 149°, has been isolated by crystallisation and chromatographic methods. Piperettine, whose properties resemble those of piperine, on hydrolysis gives piperidine and piperettic acid, $C_{14}H_{12}O_4$, a golden-yellow crystalline solid m.p. 224°. Treatment of the acid chloride with piperidine regenerates piperettine. The foregoing evidence, combined with ultra-violet absorbtion data and the fact that both piperettine and piperettic acid give positive reactions in the Labat test for the methylenedioxy group, suggested that piperettine is a vinylogue of piperine ($C_{17}H_{19}O_3N$). This conclusion was verified by the synthesis of piperettine in which piperonal was condensed with acetaldehyde to give piperonylideneacetaldehyde which on treatment with methyl γ-bromocrotonate in the presence of zinc gave methyl 7-(3:4-methylenedioxyphenyl)hepta-2:4:6-trienoate.

Hydrolysis of the ester gave 7-(3:4-methylenedioxyphenyl) hepta-2:4:6-trienoic acid identical with piperettic acid. Condensation of the chloride of the synthetic acid with piperidine gave 7-(3:4-methylenedioxyphenyl)hepta-2:4:6--trienopiperidide identical with piperettine. The stereochemistry of piperettic acid and methods of establishing its configuration by synthesis are discussed. Two new derivatives of piperine are described.

PART II. THE CONSTITUENTS OF THE NON-SAPONIFIABLE FRACTION OF SPARTIUM JUNCEUM L.

An investigation of the constituents of the non-saponifiable fractions of the wax and "essence absolute" of Spanish broom (Spartium junceum L.) is described. The main compound of the wax is found to be a long-chain paraffin, m.p. 65°, probably n-nonacosane or a mixture of n-nonacosane and n-hentriacontane, while chromatography has revealed the presence of a long-chain alcohol or mixture of alcohols m.p. 75°, together with β-amyrin and β-sitosterol as minor constituents. By chromatographic treatment on alumina the non-saponifiable fraction of the absolute has yielded the following: An unidentified sulphur-containing

oil; an unidentified substance m.p. 66°, believed to be a long-chain secondary alcohol; a complex triterpene mixture in which ∝-amyrin, β-amyrin and lupeol have been identified, a further compound isolated as its benzoate, and the presence of others suspected; an unidentified solid m.p. 69°; an unidentified solid m.p. 73°, believed to be a long-chain alcohol; an unidentified solid m.p. 57° which gives a royal blue Liebermann-Burchard colouration; β-sitosterol; a long-chain aliphatic diol, octadecane-1:18-diol, which is the first long-chain diol to be isolated from a plant source and which may have some significance with regard to the metabolism of plant products; a compound $C_{26}H_{54}O_2$, m.p. 104.5°, believed to be hexacosane-1:26--diol.

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PART II.

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PART I.

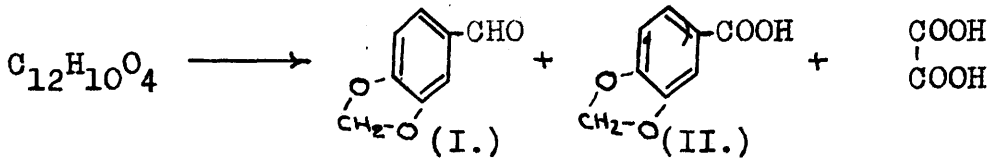
PIPERETTINE FROM *PIPER NIGRUM*; ITS ISOLATION, IDENTIFICATION, AND SYNTHESIS.

INTRODUCTION.

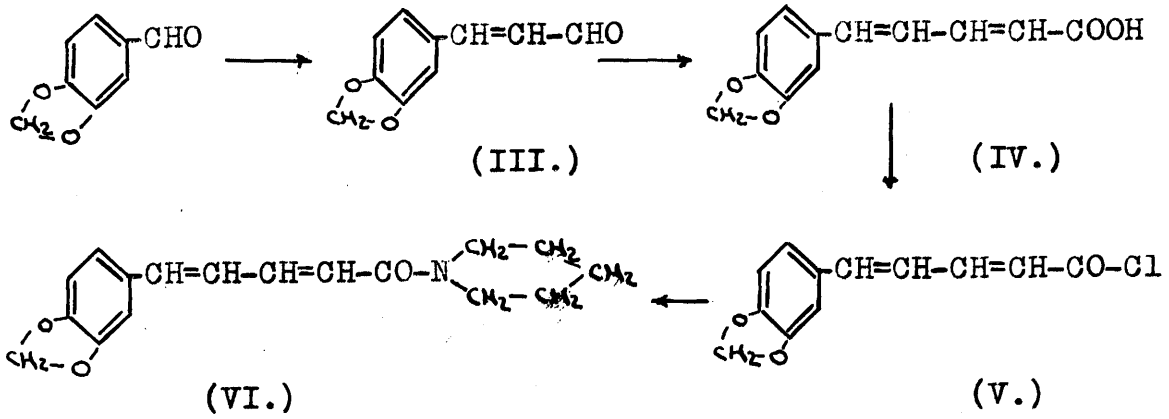
Investigations of solvent extracts of plants of the Piper species have occupied the attention of chemists for over a century, during which, several interesting substances have been isolated and their structures elucidated. The characteristic compound of the species is piperine which was first isolated by Oersted (1) in 1819 from the fruits of Piper nigrum which provide the black and white peppers of commerce. The piperine content of Piper nigrum varies from 6 - 11%. In 1879, Fluckiger and Hanbury (2) showed the substance to be present in two varieties of long pepper, Piper longum and Piper officinarum, while it has also been obtained from Ashanti black pepper, Piper clusii (3) and has recently been found in Kissi pepper, Piper farnechoni (4). In the commercial preparation of piperine, ground black pepper is extracted with ethanol, the solvent evaporated and the residue extracted with aqueous sodium hydroxide solution to remove resin (chavicine). The residue is then dissolved in hot ethanol from which crystalline piperine separates readily on cooling. An ethanolic solution of piperine has a strong pepper taste although the solid itself is tasteless (5).

In the elucidation of the structure of piperine, Regnault (6) in 1838 established the formula as $C_{17}H_{19}O_3N$.

Degradative evidence was provided by Anderson (7) and Babo and Keller (8) who hydrolysed piperine and observed the formation of piperidine and an acid, $C_{12}H_{10}O_4$, which was named piperic acid. In 1882, Rugheimer (9) regenerated piperine from piperoyl chloride and piperidine thus proving it to be the piperidide of piperic acid. The structure of piperic acid was deduced from oxidative experiments. The acid, on oxidation with potassium permanganate, furnished oxalic



acid together with two new products piperonal and piperonylic acid (10) whose structures were subsequently established (11) as (I) and (II) respectively. From the foregoing evidence and from the fact that piperic acid was known to contain two double bonds, the structure was postulated as (IV). This view was confirmed by the synthesis (12) of piperic acid in which piperonal was condensed with acetaldehyde to give piperonylidene--acetaldehyde (III) which was converted to (IV) by the

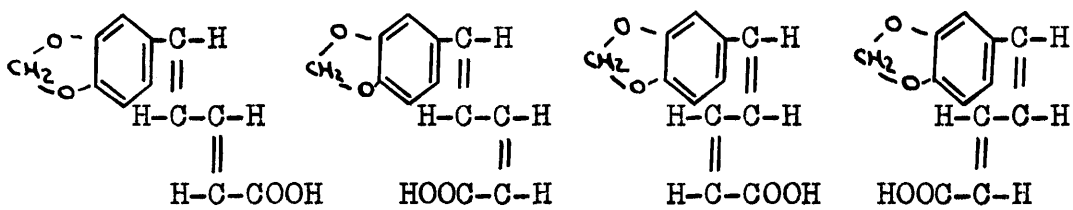


action of sodium acetate and acetic anhydride. The above evidence, in conjunction with Rugheimer's partial synthesis of piperine (9) established the structure of the latter as (VI).

Systematic variations of the piperine molecule have shown (5,13) that the sharp, pepper-like taste of an ethanolic solution of piperine is dependent on the presence of the phenyl group, the 4-carbon chain and the amide linkage, but is independent of the methylenedioxy group, the double bonds and the piperidine nucleus.

Accompanying piperine in black pepper is a brown resin which was investigated in 1876 by Bucheim (14) and named chavicine. Chavicine is readily soluble in ether and use is made of this property to effect a separation (15) from the sparingly soluble piperine present in the

crude pepper extract. By the hydrolysis of chavicine Bucheim obtained piperidine and an uncrystallisable acid, chavicinic acid, which he did not investigate further. The work was taken up by Ott and his colleagues (15,16) who on hydrolysis of chavicine obtained piperidine and isochavicinic acid, $C_{12}H_{10}O_4$, the latter being formed by rearrangement of chavicinic acid. Isochavicinic acid is isomeric with piperic acid and since catalytic hydrogenation of both acids provides the same tetrahydro derivative Ott concluded that isochavicinic acid is a geometric isomer of piperic acid. The four possible stereochemical forms of piperic



(VII.) (VIII.) (IX.) (X.)

acid can be represented by structures (VII) - (X) in which the trans-trans form (VII) corresponds to piperic acid itself and the cis-trans form (VIII) to isopiperic acid prepared by the condensation of piperonylideneaceta--ldehyde with malonic acid followed by decarboxylation of

the product. The cis-cis (IX) and the trans-cis (X) forms were deduced by Ott to correspond to chavicinic and isochavicinic acids respectively. The conclusion was reached that chavicine is the piperidide of isochavicinic acid. A comprehensive synthetic study of the isomeric piperic acids has been reported by Lohaus (17). It is interesting to note that Bucheim (14), Ott (15,16) and others held the view that chavicine and not piperine is the active principle of black pepper.

Other substances obtained from Piper plants include piperovatine which was isolated in 1895 by Dunstan and Garnet (18) from an extract of Piper ovatum. When hydrolysed with water, piperovatine yielded a volatile base, probably a pyridine derivative, together with an oil having the odour of anisole. The authors promised a further communication on piperovatine but this did not appear.

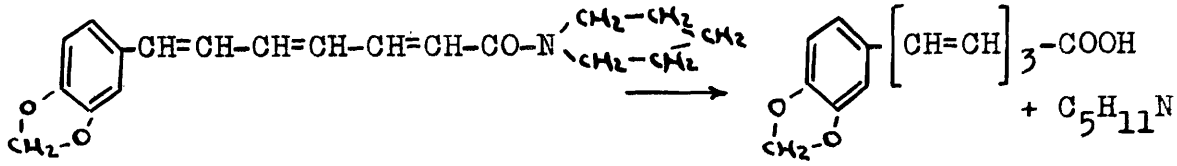
Recently, members of the firm of T. and H. Smith, Edinburgh, engaged in the isolation of piperine from an ethanolic extract of black pepper, suspected the presence of a second solid component in the concentrated mother liquors. This component was named piperettine and it was observed that it could be separated from piperine only with great difficulty. Evaporation of the solvent from the mother liquors gave a yellow-brown solid which was

obviously a mixture of piperine and piperettine with the presence of chavicine also probable. A quantity of this piperettine-containing material was kindly supplied to the Royal Technical College and the following pages describe its investigation.

THEORETICAL.

The isolation of pure piperettine from the crude solid recovered from the ethanolic mother liquors of the Piper nigrum extract was accomplished by two methods. In the first, a solution of the solid in boiling ethyl acetate was allowed to cool slowly in an insulated vessel. Under ideal conditions, large aggregates of piperine and piperettine were formed which were easily distinguishable from each other and permitted a ready separation by hand. The second method involved the use of liquid chromatography. Using alumina as adsorbent, the early fractions contained piperine in a fairly pure state. A series of fractions was then collected in which there was a gradual transition from piperine-rich to piperettine-rich mixtures, followed, on continued elution with a more powerful eluent, by a small final fraction of pure piperettine. It had been hoped that chromatography would reveal the presence of still further compounds in the extract, but apart from a brown resin (probably chavicine) which contaminated most of the fractions no indication of other substances was found. Piperettine, $C_{19}H_{21}O_3N$, m.p. 146 - 149°, separates from ethanol in clusters of pale yellow-green, highly refracting needles and its properties bear a marked similarity to those of piperine. It is sparingly soluble in dilute acids and

alkalis and with concentrated hydrochloric acid gives a bright yellow solution while with concentrated sulphuric acid a blood-red colouration is produced. It gives a positive reaction in the Labat (19) test for the methylenedioxy group and its ultra-violet absorbtion spectrum in ethanol solution shows a broad band with a maximum at 3640A. whereas that of piperine exhibits a similar broad band with a maximum at 3450 A. (Fig. 1). The above evidence together with the fact that it is more strongly adsorbed on alumina than is piperine, seemed sufficient ground to ascibe to piperettine the structure (XI) which differs from that of piperine (VI)

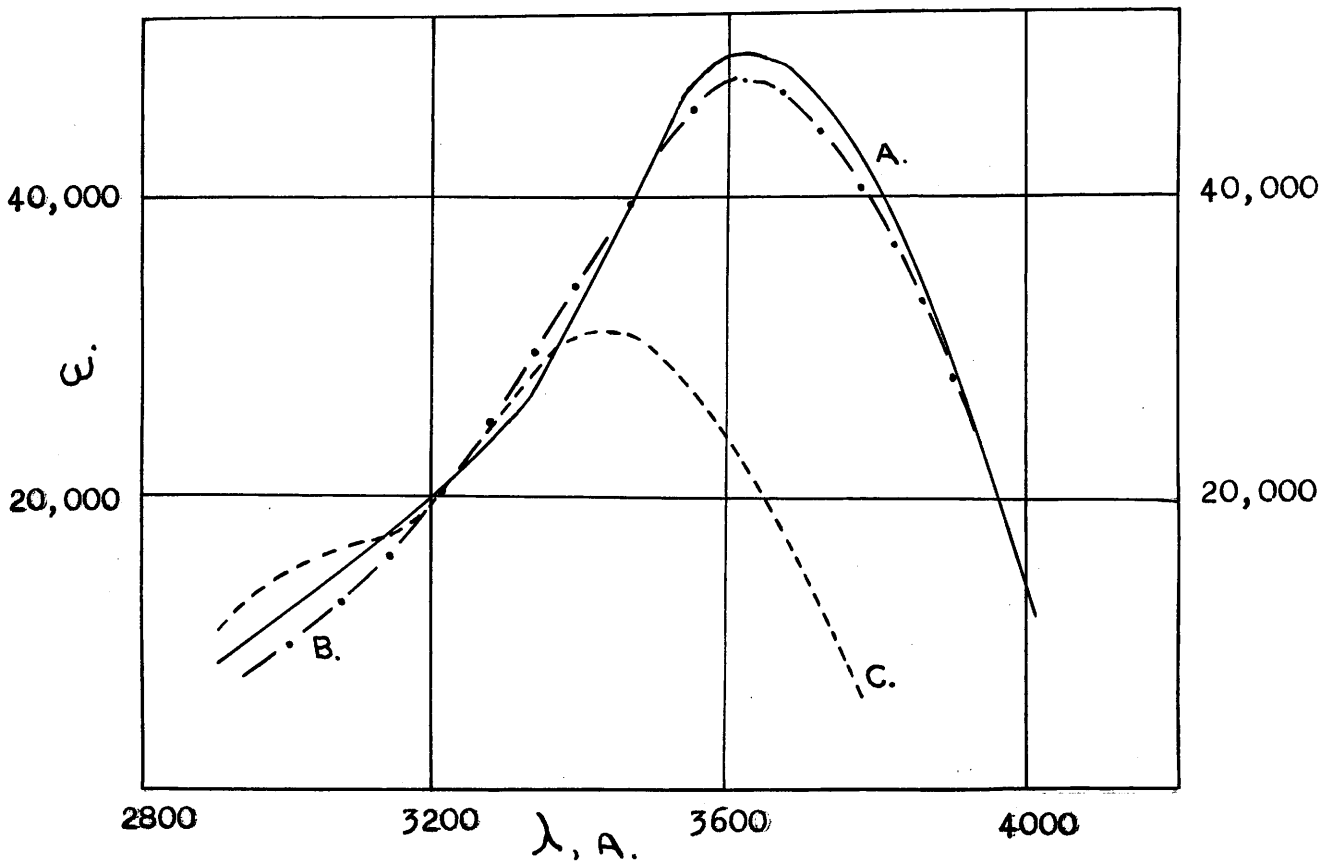


(XI.) (XII.)

only by virtue of an additional -CH=CH- group in the conjugated side chain.

To provide degradative evidence in support of (XI) piperettine was hydrolysed with 20% ethanolic potassium hydroxide under conditions similar to those used by Babo and Keller (8) for the hydrolysis of piperine. Two products were isolated from the hydrolysis mixture. The

Fig. 1.

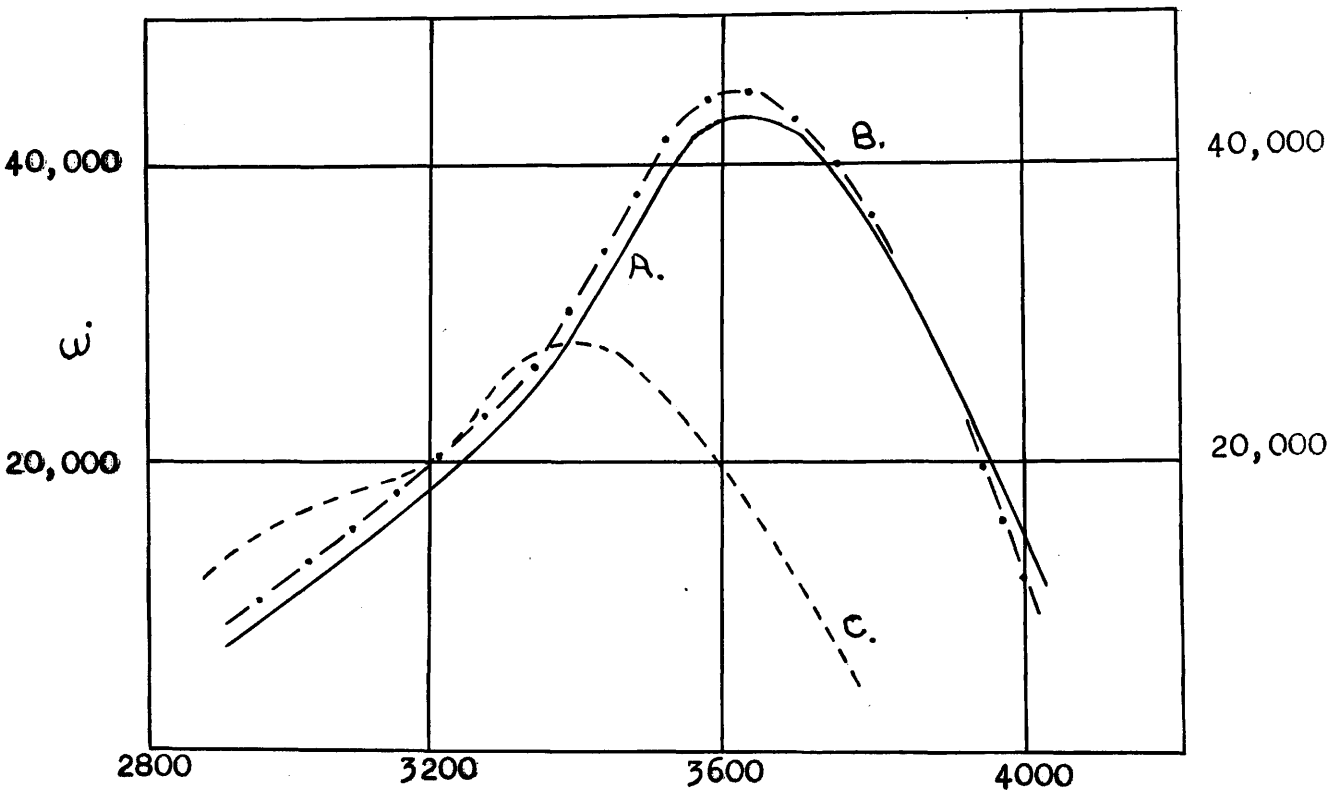


A. Piperettine.

B. 7-(3:4-Methylenedioxyphenyl)hepta-2:4:6-trienopiperidide.

C. Piperine.

Fig. 2.



A. Piperettic Acid.

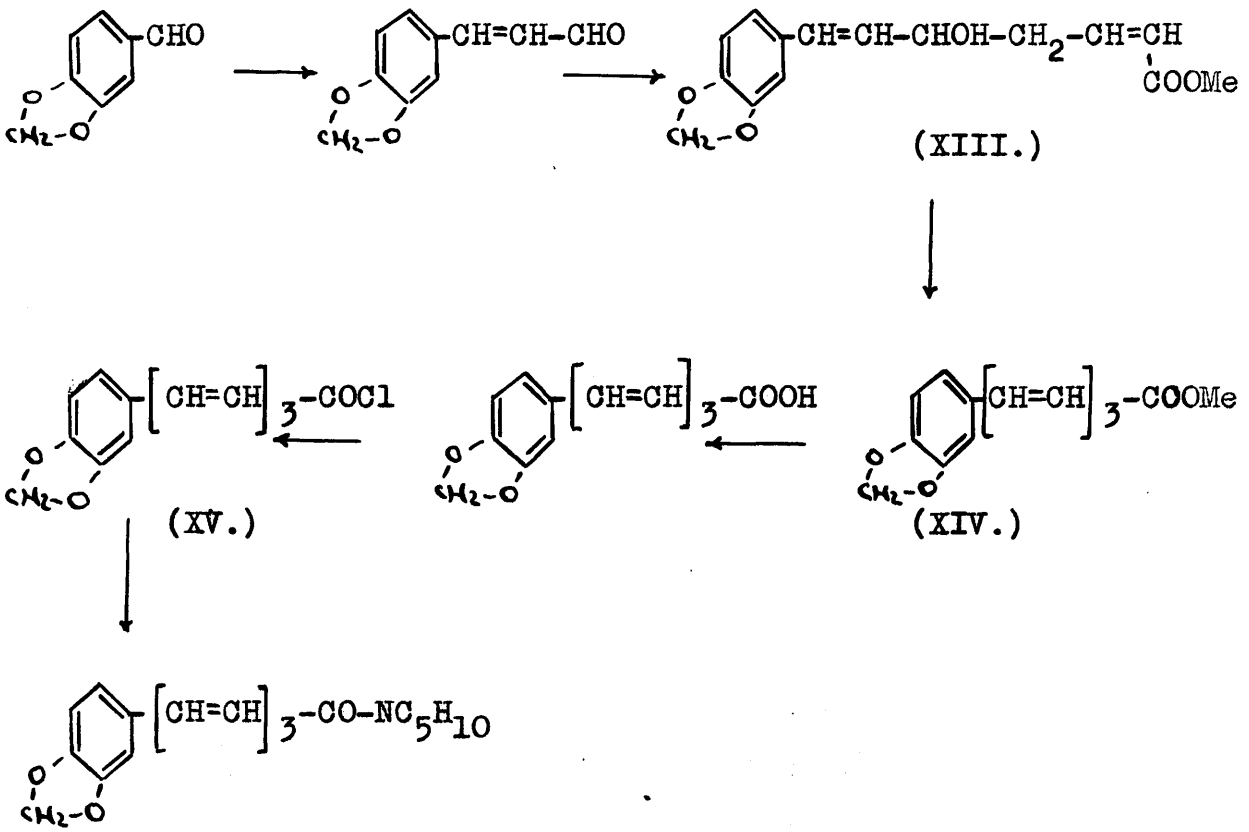
B. 7-(3:4-Methylenedioxyphenyl)hepta-2:4:6-trienoic Acid.

C. Piperic Acid.

first was a golden-yellow crystalline acid, $C_{14}H_{12}O_4$, m.p. 224°, for which the name piperettic acid is proposed and which, on the postulated structure for piperettine is 7-(3:4-methylenedioxyphenyl)hepta-2:4:6--trienoic acid (XII). Piperettic acid was characterised by the formation of a methyl ester. In ethanol solution, piperettic acid exhibits an absorbtion maximum at 3600 A. whereas piperic acid exhibits a maximum at 3400 A. (Fig. 2). The second hydrolysis product was a liquid base which was identified as piperidine by the preparation of its hydrochloride, benzenesulphonamide and picrate and comparison of these with authentic specimens. The piperidine isolated was equivalent to 77% of that theoretically contained in piperettine.

Confirmation that piperettine is indeed the piperide of piperettic acid was obtained by regenerating piperettine from its hydrolysis products. For this, a suspension of the acid in benzene was heated with excess thionyl chloride for 4 hours at 80°. The reaction proceeded smoothly to give the acid chloride as an orange solid which, without further purification was reacted in benzene solution, at room temperature, with excess piperidine. The product was identical with piperettine.

The obvious starting material for the total synthesis of piperettine was piperonal which was condensed with acetaldehyde under conditions described by Lohaus (17)



to give piperonylideneacetaldehyde in 15% yield. Several alternative methods were available for the conversion of this compound to methyl 7-(3:4-methylenedioxyphenyl)hepta-

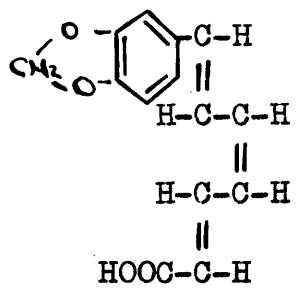
-2:4:6-trienoate (XIV) but nearly all required a further aldol-type condensation with all the inherent difficulties associated with that type of reaction. A Reformatsky reaction seemed to hold more promise of success and piperonylideneacetaldehyde was therefore condensed, in presence of zinc foil, with methyl γ-bromocrotonate to give (XIV) in very poor yield (9%) despite the fact that most of the zinc had dissolved. A possible explanation of this behaviour, which is common in Reformatsky reactions, has been put forward by Jones, O'Sullivan and Whiting (20). In addition to the unsaturated ester (XIV) a brown oil was recovered from the reaction which, it was thought, might contain some of the intermediate hydroxy-ester (XIII). Attempts to dehydrate the oil, first by high vacuum distillation and then by the action of anhydrous oxalic acid, yielded no solid product.

Hydrolysis of (XIV) with 10% ethanolic potassium hydroxide gave a quantitative yield of 7-(3:4-methylene--dioxyphenyl)hepta-2:4:6-trienoic acid m.p. 224° alone and when mixed with piperettic acid. The ultra-violet absorbtion spectra of the synthetic acid and of piperettic acid are shown in Fig. 2.

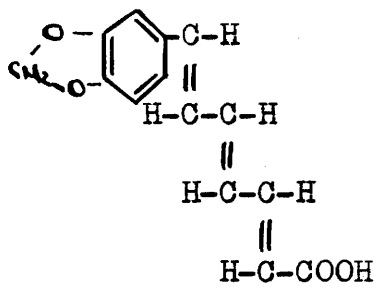
The final stage in the synthesis was accomplished by treating a suspension of the trienoic acid in benzene

with excess thionyl chloride under conditions identical with those used for the regeneration of piperettine from its hydrolysis produots. The resulting acid chloride (XV) on treatment with piperidine gave 7-(3:4-methylenedioxyphenyl)hepta-2:4:6-trienopiperidide which was identical with piperettine. The ultra-violet absorbtion spectra of the natural and synthetic materials are given in Fig. 1.

The foregoing experiments, while confirming that piperettine is the piperidide of 7-(3:4-methylenedioxy--phenyl)hepta-2:4:6-trienoic acid, give no information on the stereochemical arrangements in the acid molecule. Since there are three double bonds present it is possible for the acid to exist as any of eight geometric isomers. The problem is simplified, however, since piperonylidene--acetaldehyde is known to possess a trans configuration (15). Also, since methyl γ-bromocrotonate was prepared from methyl crotonate which has a trans configuration it seems reasonable to assume that no rearrangement takes place on bromination and that the configuration of the bromoester is also trans . If the above assumptions are made, the two possible structures for piperettic acid are represented by (XVI) and (XVII) in which the stereochemical arrangements are trans-cis-trans and



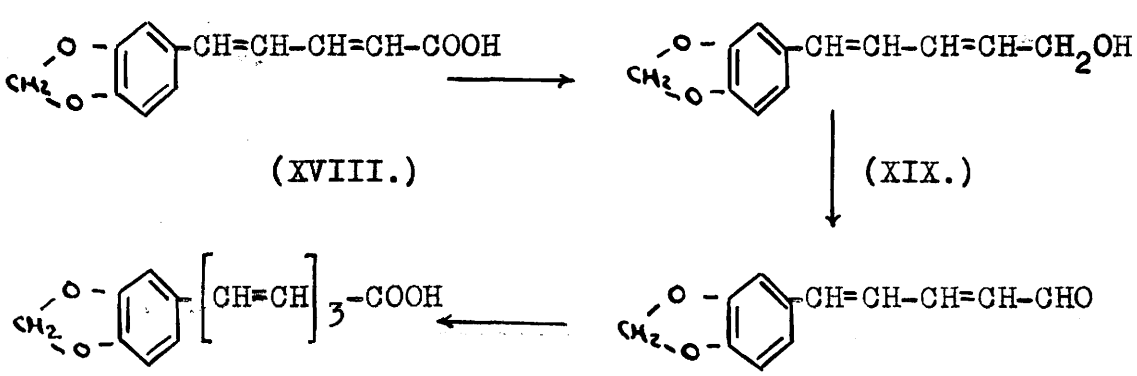
(XVI.)



(XVII.)

trans-trans-trans respectively. The establishment of the configuration of the acid by an unambiguous synthetic method presents many difficulties since almost every possible synthesis involves the formation of a new double bond, the stereochemical arrangement around which cannot be predicted. A,B and C represent possible routes to trienoic acids of known configurations. In route A,

A.



(XVIII.) (XIX.) (XX.) (XXI.)

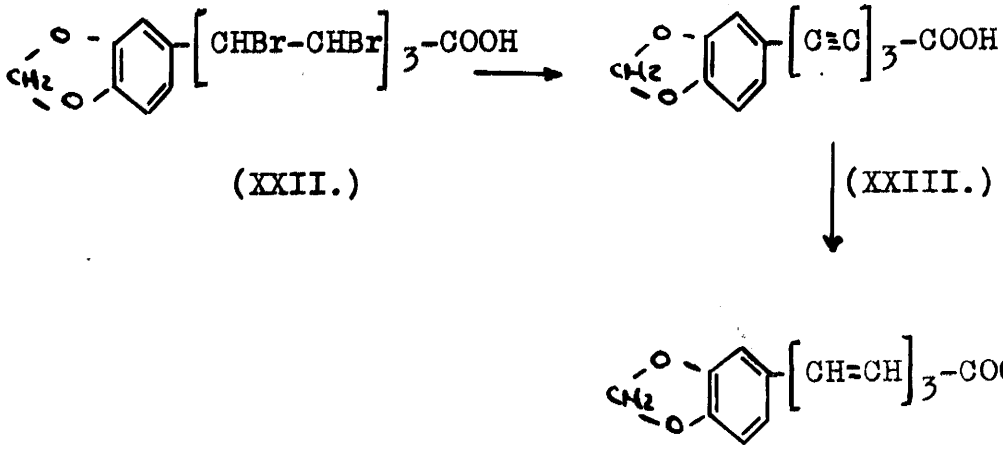
piperic acid (XVIII) which has a trans-trans configuration is reduced by means of lithium aluminium hydride (21) to

the primary alcohol (XIX) which under mild oxidation with tertiary butyl chromate (22) or with potassium dichromate and sulphuric acid at low temperature (23) should give piperic aldehyde (XX)(24). Condensation of the aldehyde with malonic acid in presence of pyridine followed by decarboxylation, or alternatively with bromoacetic ester in presence of zinc followed by hydrolysis, should provide an acid (XXI) in which the configuration of the double bond adjacent (α-β) to the carboxyl group is the only unknown. If this acid is identical with piperettic acid then the latter must have a trans-trans-trans structure. If the acids are not identical then either piperettic acid has a cis configuration at the middle (γ-δ) double bond or (XXI) has a cis configuration at the bond (α-β) formed in the synthesis. If the above synthesis did not furnish an acid identical with piperettic acid the sequence of reactions could be repeated using isopiperic acid, which has a cis-trans configuration (c.f. p.4), as starting material. In this way an acid would be obtained having a cis arrangement at the middle (γ-δ) double bond. If this acid were identical with piperettic acid then the latter would possess a trans-cis-trans structure. If the acids were not identical then the bond (α-β) formed

during the synthesis must have acquired a cis configuration.

Routes B and C involve the application of acetylene chemistry (25). In B, piperettic acid is

B.

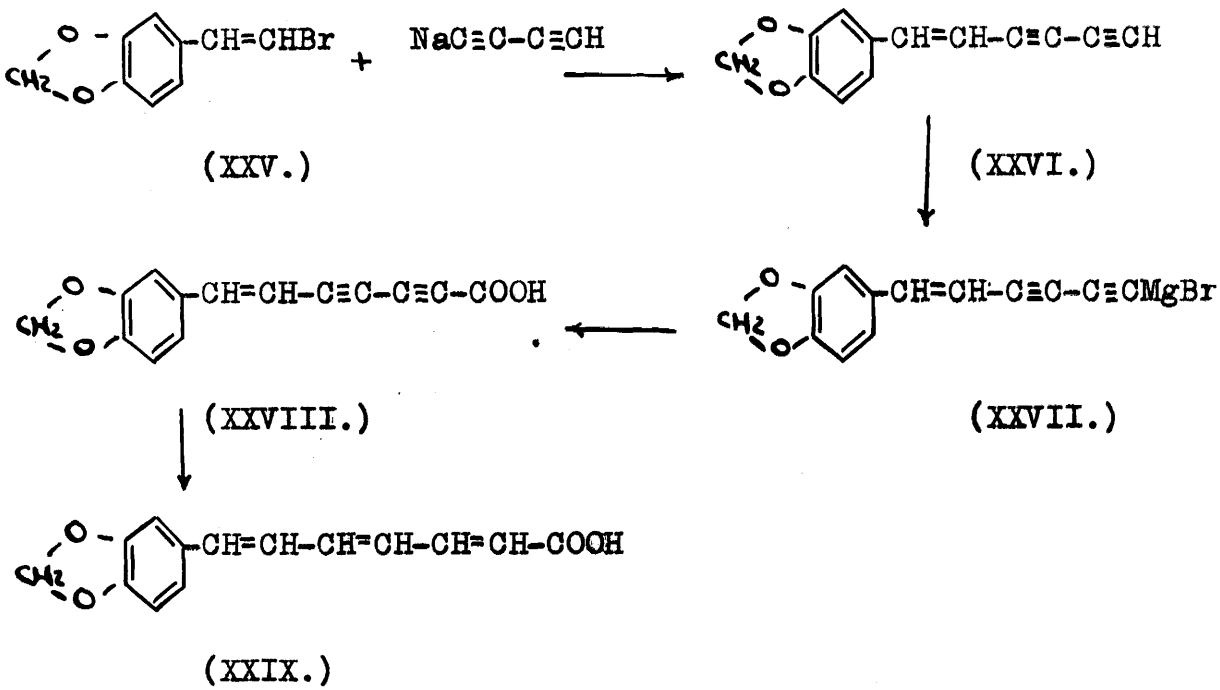


(XXII.) (XXIII.) (XXIV.)

treated with bromine to give a hexabromo derivative (XXII) which on dehydrohalogenation with sodamide in liquid ammonia (26) should furnish the triacetylenic acid (XXIII). This, on partial reduction with sodium in liquid ammonia should give a piperettic acid (XXIV) with a trans-trans--trans structure for comparison with the natural product.

Route C has as starting material, 3:4-methylenedioxy--ω-bromostyrene (XXV)(17) which is reacted with monosodiodiacetylene (25,28) to give (XXVI) which on

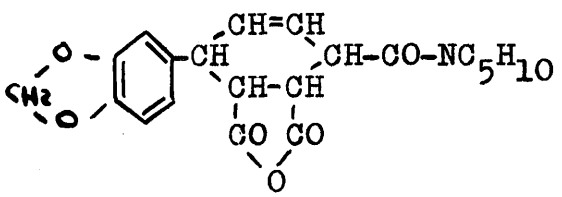
C.



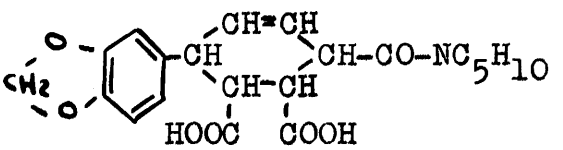
treatment with ethyl magnesium bromide should give (XXVII) which with carbon dioxide furnishes the diacetylenic acid (XXVIII). Partial reduction of this acid with sodium in liquid ammonia would give for comparison, an acid (XXIX) having <u>trans</u> configurations at the α-β and γ-δ double bonds. The configuration of the remaining bond is unknown.

During the early stages of the work on the isolation of piperettine, before any success had been obtained by physical methods of separation and before any knowledge

of the structure of the substance was available, it had been intended to use the presence of a conjugated system of double bonds in piperine to remove it from the mixture as a maleic anhydride adduct. Work on this had only reached the preliminary stage when the isolation of piperettine by other means rendered it unnecessary. Since, however, both the adduct (XXX) and the dicarboxylic acid



(XXX.)



(XXXI.)

(XXXI) formed by opening the anhydride ring are new compounds an account of their preparation is given in the present work. The isolation of piperettine represents a further addition to the group of naturally occurring amides which includes piperine, chavicine, capsaicin (29), spilanthol (30), pellitorine (31), herculin (32), fagaramide (33) and affinin (34). Some members of the group have received attention as insecticides.

EXPERIMENTAL.

The analytical results for new compounds are given in the form: "Found: ——— . ——— requires ——— ."

For all other compounds the results are given in the form: "Found: ——— . Calc. for ——— : ——— ."

After the removal of several crops of piperine from an ethanolic extract of the crushed fruits of Piper nigrum an amorphous yellow powder m.p. 100 - 112° was obtained from the mother liquors.

Isolation of Piperettine.- (a) By hand-picking. A solution of the solid, m.p. 100 - 112° (6.9g.) in boiling ethyl acetate (11 c.c.) was cooled over a period of 1 hour to 30°. The crystals which had then separated, being homogeneous, the mother liquor was removed by decantation and the residual solid (1.3g., m.p. 117 - 127°) crystallised twice from ethyl acetate to give piperine (0.9g.) as pale yellow rods m.p. 129° undepressed when mixed with a genuine specimen (m.p. 129°). The decanted mother liquor, on standing, deposited two different types of crystalline aggregates; the first as large, leaf-shaped, semi-transparent pale yellow clusters and the other as spherical, opaque, wart-like deposits. The size of the deposits permitted a ready separation by hand-picking. After two crystallisations from ethyl acetate, the leaf-shaped clusters (2.25g.) yielded piperine as pale yellow highly refracting rods m.p. 129°. The opaque spheroids (1.33g.) on repeated crystallisation from ethyl acetate gave piperettine as yellow-green blades

m.p. 146 - 149°.

Found: C, 73.6; H, 7.1; N, 4.7.
C, 73.5; H, 6.9; N, 4.5.
C, 73.4; H, 6.9.

$C_{19}H_{21}O_3N$ requires C, 73.3; H, 6.8; N, 4.5%.

(b) By chromatography. A solution of the solid m.p. 100 - 112° (10g.) in benzene-petrol (b.p. 40 - 60°)(3:1; 200 c.c.) was passed down a tower (30 x 5 cm.) of activated alumina (Brockmann II) and the chromatogram developed with benzene-petrol and benzene when it had the following appearance. At the foot of the column there was a colourless region (zone A) surmounted by a narrow yellow band (zone B) which was separated by a colourless band (zone C) from the main band (zone D). Zone D was pale yellow at the bottom and became progressively darker towards the top. A colourless band (zone E) separated the main band from two narrow brown bands (zone F) while at the extreme top of the tower was a narrow colourless zone (G). The results of prolonged elution with various solvents are given in Table 1.

Fraction 1, on recrystallisation from ethyl acetate gave piperine as pale yellow rods m.p. 127 - 130°. Similarly, fraction 2 gave piperine as almost colourless

Table 1.

"Petrol" refers to light petroleum b.p. 40 - 60°.

Fraction.	Zone.	Solvent.	Wt. of Fraction.	Description.
1	A	Benzene-petrol (3:1, 7.6 l.) (5:1, 1.5 l.) Benzene (2.1 l.)	0.34g.	Sticky yellow solid.
2	B	Benzene (1.8 l.)	0.39g.	Pale yellow solid m.p. 127 - 130°.
3	C	Benzene (1.5 l.)	1.72g.	Yellow solid m.p. 126 - 129° after one cryst. from ethyl acetate
4	D (1st. quarter)	Benzene (1.5 l.)	1.75g.	Brown resin.
5	D (2nd. quart.)	Benzene (1.5 l.)	1.70g.	Brown resin.
6	D (3rd. quart.)	Benzene (1.5 l.)	1.65g.	Brown resin.
7	D (4th. quart.)	Benzene (3.5 l.)	1.00g.	Brown resin.
8	E	Benzene-ethanol (99:1, 0.4 l.) (98:2, 0.5 l.)	0.10g.	Brown resin.
9	F	Benzene-ethanol (95:5, 0.6 l.)	0.50g.	Brown resin.
10.	-	Benzene-ethanol (1:1, 0.6 l.)	0.05g.	Brown resin.

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rods m.p. 128 - 130°. In each case the m.p. was undepressed when the sample was mixed with authentic piperine. Crystallisation of fractions 3 and 4 gave a slightly less pure piperine. Fraction 5, on crystallisation from ethyl acetate deposited a crop of piperine (0.60g.) m.p. 124 - 128° followed by a crop of material which on recrystallisation from ethyl acetate gave piperettine (0.12g.) as prisms m.p. 138 - 146° undepressed when mixed with the specimen isolated by hand picking. A hot saturated solution of fraction 6 in ethyl acetate, when cooled slowly, deposited two distinctly different sets of crystalline aggregates which were separated by hand in the usual manner and recrystallised from ethyl acetate to give piperine (0.11g.) as rods m.p. 124 - 128° and piperettine (0.10g.) as prisms m.p. 142 - 146°. Fraction 7 when crystallised from ethyl acetate gave a deposit (0.29g.) of the spherical aggregates characteristic of impure piperettine which, after three crystallisations from ethanol yielded piperettine (0.15g.) as pale yellow-green highly refracting needles m.p. 141 - 147°. Crystallisation of fraction 9 from ethyl acetate gave a crop (0.03g.) of prismatic plates which on recrystallisation from the same

solvent provided piperettine as prisms m.p. 144 - 147°. No crystalline material was recovered from fractions 8 and 10. The combined crops of piperine from fractions 1 - 6 were recrystallised from ethyl acetate to give pure piperine (2.0g.) as pale yellow rods m.p. 129°. Similarly, recrystallisation of the combined piperettine crops from fractions 5, 6, 7 and 9 from the same solvent gave the substance as pale yellow-green prisms m.p. 145°(0.21g.).

Piperettine dissolves readily in chloroform, acetone and glacial acetic acid, is sparingly soluble in ethanol, ethyl acetate, benzene and ether and is insoluble in water, dilute acids and alkalis. It is sparingly soluble in concentrated hydrochloric acid giving a bright yellow solution while with concentrated sulphuric acid it gives a blood-red colour. In the Labat test for the methylenedioxy group piperettine gives colour changes of red → brown → olive green → emerald green, the corresponding colour sequence for piperine being red → brown → green → blue. Piperettine crystallises as pale yellow-green blades or prisms from ethyl acetate and as rosettes of needles from ethanol.

Hydrolysis of Piperettine.- Isolation of Piperettic Acid. A solution of piperettine (2.0g.) in absolute ethanolic potassium hydroxide (30 c.c.; 20%) was refluxed on a steam bath for 6 hours. The crystalline potassium salt which separated during this period was collected, washed with ethanol (10 c.c.) and dried in vacuo. The dry salt (1.6g.) was dissolved in hot water (1600 c.c.), the solution acidified with hydrochloric acid (1.2 c.c., d.1.16 in 4 c.c. water) and the voluminous bright yellow precipitate collected, washed thoroughly with water, dried (1.3g.) and thrice crystallised from ethanol to yield piperettic acid as golden-yellow felted needles m.p. 223 - 224°.

Found: C, 68.7; H, 5.1.

$C_{14}H_{12}O_4$ requires C, 68.9; H, 4.9%.

The methyl ester, prepared by the action of diazomethane at room temperature on an ethereal suspension of the acid, crystallised from ethanol as brittle, orange-yellow needles m.p. 174°.

Found: C, 69.8; H, 5.3.

$C_{15}H_{14}O_4$ requires C, 69.8; H, 5.4%.

Identification of Piperidine as a Hydrolysis Product.

(a) As the hydrochloride. The ethanolic potassium

hydroxide filtrate and washings from the above isolation of potassium piperettate were combined and the ethanol removed under slightly reduced pressure, the distillate being collected in a receiver cooled by an ice-salt freezing mixture. The distillate, which was strongly basic, was saturated with hydrogen chloride and evaporated to dryness to give a colourless solid (0.6g.) which on crystallisation from ethanol gave piperidine hydrochloride as long, colourless prismatic needles m.p. 244° alone or mixed with an authentic specimen.

(b) As the benzenesulphonamide. A portion of the foregoing hydrochloride was dissolved in dilute caustic soda, shaken for 30 minutes with excess benzenesulphonyl chloride and the mixture poured into water. The colourless solid which separated was collected and recrystallised from ethanol from which benzenesulphono--piperidide was obtained as prismatic needles m.p. 91° alone or mixed with an authentic specimen.

(c) As the picrate. A portion of the hydrochloride was dissolved in a little hot water and treated with a cold, saturated aqueous solution of picric acid. A turbidity developed, followed by the gradual separation of long, fine, yellow needles which on recrystallisation from water gave piperidine picrate m.p. 150° alone or

mixed with an authentic specimen.

Regeneration of Piperettine.- Piperettic acid (0.66g.) in suspension in dry benzene (30 c.c.) was treated with pure thionyl chloride (4 c.c.) and the mixture heated for 4 hours at 80°. A steady reaction set in immediately and the acid dissolved with evolution of sulphur dioxide and hydrogen chloride. The bright orange solid chloride, obtained on removal of the benzene and excess thionyl chloride under vacuum (bath temperature 30°), was dissolved in dry benzene (10 c.c.) and treated with a solution of piperidine (2 c.c.) in dry benzene (5 c.c.). The mixture warmed slightly and deposited a colourless solid. When the mixture had stood at room temperature for 18 hours the solid was collected (0.31g.) and after two crystallisations from ethanol was obtained as long needles m.p. 244° undepressed when mixed with authentic piperidine hydrochloride. The benzene filtrate obtained after removal of the hydrochloride was diluted to ca. 80 c.c. with benzene and the resulting solution washed successively with 3N hydrochloric acid, water, dilute sodium carbonate and again with water. The residue (0.69g.) obtained on removal of the solvent from the dried (sodium sulphate) solution was recrystallised once from ethyl acetate and repeatedly from ethanol from which it separated

as pale yellow-green needles m.p. 143 - 146°, undepressed on admixture with piperettine.

Piperonylidineacetaldehyde.- (Lohaus, 17). A mixture of piperonal (50g.), acetaldehyde (75g.), water (2500 c.c.) and sodium hydroxide (50g. of 10%) was stirred at room temperature in an atmosphere of nitrogen for 5 days. When the experiment had proceeded for 2⅓ days a further quantity (75g.) of acetaldehyde was added to compensate for losses by evaporation. At the end of the stirring period, the reaction mixture, which was bright yellow and contained a quantity of yellow-brown oil in suspension, was extracted with ether (10 x 200 c.c.) and the extract dried over calcium chloride. The brown-red viscous residue, obtained on evaporation of the ether was distilled under vacuum in an atmosphere of nitrogen. Piperonylidene--acetaldehyde distilled at 140°/ 0.4 mm. and was collected as a viscous orange liquid which partially solidified on standing. The oil contaminating the solid was removed by suction and the residue recrystallised from ethanol:petrol (1:1) to give the pure aldehyde as large, pale yellow prisms m.p. 84° (Lohaus gives m.p. 84°). The yield (8.0g.) allowing for recovered piperonal (6.0g., b.p. 86 - 98°/ 0.3 - 0.4 mm.) was 15% of the theoretical.

Methyl γ-Bromocrotonate.- (Ziegler, 35). A mixture of N-bromosuccinimide (50g. of 90%; 1 mole) and methyl crotonate (51g.; 2moles) in carbon tetrachloride (80 c.c.) was refluxed on a water bath for 26 hours when a test with acidified potassium iodide solution showed the reaction to be complete. The deposited succinimide was collected, washed with carbon tetrachloride (30 c.c.) and the filtrate and washings fractionated through a packed column. After the removal of carbon tetrachloride and excess methyl crotonate, methyl γ-bromocrotonate distilled as a pale yellow mobile liquid which was collected at 87°/10 mm. Yield 40g. (88% theory).

Methyl 7-(3:4-Methylenedioxyphenyl)hepta-2:4:6--trienoate.- Piperonylideneacetaldehyde (7.5g.; 1 mole) was dissolved in pure dry, thiophen-free benzene (35 c.c.) and treated, with stirring, with zinc shavings (2.8g.) and methyl γ-bromocrotonate (7.6g.; 1 mole). The reaction was initiated by the addition of a trace of iodine and by gentle warming. When the first vigorous reaction had subsided (10 - 15 minutes) the mixture was heated under reflux for 2 hours during which a brown gelatinous solid separated and the mixture was diluted with benzene (10 c.c.) to ease the stirring. When the reaction mixture had

cooled to room temperature it was diluted with benzene (100 c.c.) and the resulting suspension decanted from the unreacted zinc and shaken with 3N hydrochloric acid (60 c.c.). The benzene layer was then washed successively with water, dilute sodium carbonate and again with water and dried over sodium sulphate. After evaporation of the solvent, the sticky orange-brown residue was triturated with ether (15 c.c.) and the solid collected and washed with ether (5 c.c.) to give a yellow solid (0.9g.) m.p. 170°. On slow evaporation of the ether filtrate and washings a further quantity (0.1g.) of the same material was obtained. The combined products were recrystallised from benzene to give methyl 7-(3:4-methylenedioxyphenyl) hepta-2:4:6-trienoate as yellow prismatic needles m.p. 174° undepressed when mixed with methyl piperattate.

Found: C, 69.7; H, 5.7.

$C_{15}H_{14}O_4$ requires C, 69.8; H, 5.4%.

7-(3:4-Methylenedioxyphenyl)hepta-2:4:6-trienoic Acid.- Methyl 7-(3:4-methylenedioxyphenyl)hepta-2:4:6-trienoate (0.25g.) in absolute ethanol (22.4 c.c.) containing potassium hydroxide (2g.) was refluxed for 3 hours. The silky precipitate was then collected, washed with ethanol (5 c.c.), dissolved in hot water (800 c.c.)

and the solution acidified with 3N hydrochloric acid. The resulting yellow precipitate was collected, washed with water and dried to give a yellow solid (0.24g.) which on recrystallisation from ethanol yielded 7-(3:4-methylenedioxyphenyl)hepta-2:4:6-trienoic acid as light golden-yellow needles m.p. 224° alone and when mixed with piperettic acid.

Found: C, 68.8; H, 4.8.

$C_{14}H_{12}O_4$ requires C, 68.9; H, 4.9%.

7-(3:4-Methylenedioxyphenyl)hepta-2:4:6-trienopiper--idide.- A suspension of the foregoing acid (0.4g.) in dry benzene (20 c.c.) containing thionyl chloride (3 c.c.) was heated for 4 hours at 80°. The reaction proceeded in a manner exactly similar to that already described for the regeneration of piperettine from its hydrolysis products (p. 24). On working up by the same method, a yellow-brown solid (0.45g.) was obtained which was crystallised repeatedly from ethanol (charcoal) to give 7-(3:4-methylene-dioxyphenyl)hepta-2:4:6-trienopiperidide as clusters of pale yellow-green needles m.p. 146° undepressed when mixed with piperettine.

Found: C, 73.2; H, 6.5; N, 4.3.

$C_{19}H_{21}O_3N$ requires C, 73.3; H, 6.8; N, 4.5%.

Piperine Maleic Anhydride Adduct.- A mixture of piperine (2.9g.), maleic anhydride (2.0g., 100% excess) and benzene (8 c.c.) was refluxed for 20 hours. The resulting dark brown solution, on standing, deposited a crystalline solid which was collected (1.24g.) and recrystallised from benzene+ethyl acetate (1:1) to give piperine maleic anhydride adduct as colourless rectangular plates m.p. 169 - 170°.

Found: C, 65.7; H, 5.9.

$C_{21}H_{21}O_6N$ requires C, 65.9; H, 5.5%.

Di-Acid from Piperine Maleic Anhydride Adduct.- The benzene filtrate after removal of the above adduct was evaporated to dryness, the gummy residue heated for 5 - 10 minutes at 100° with sodium hydroxide (30 c.c.; 4%) and the insoluble matter collected, washed with water and dried (0.42g., m.p. 110°). On recrystallisation from ethyl acetate, the material furnished piperine as pale yellow rods m.p. 128° alone and when mixed with an authentic sample. Acidification of the alkaline filtrate after removal of the piperine gave a colourless precipitate which was collected, washed with water and recrystallised from aqueous ethanol from which the di-acid separated as small colourless prisms (0.9g.) m.p. 190°.

Found: C, 62.6; H, 5.8; N, 3.8.

$C_{21}H_{23}O_7N$ requires C, 62.8; H, 5.7; N, 3.5%.

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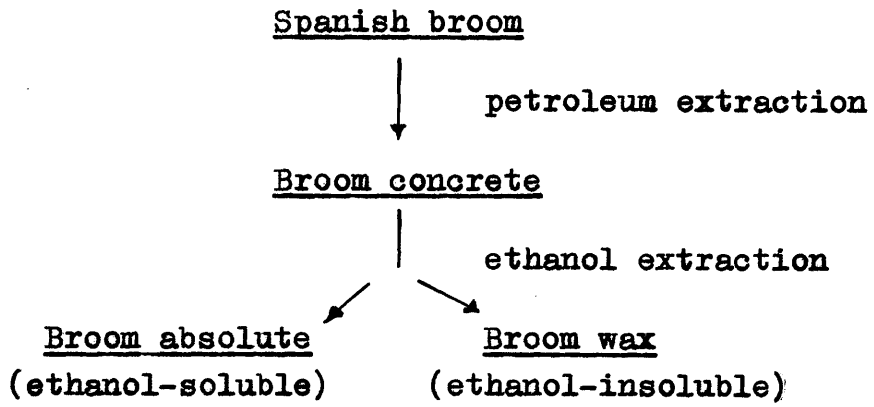
PART II.

THE CONSTITUENTS OF THE NON-SAPONIFIABLE FRACTION OF *SPARTIUM JUNCEUM* L.

INTRODUCTION.

Spanish broom (Spartium junceum L.) is a bush that grows spontaneously in the stony regions of central France and is also cultivated in certain districts, notably around Lodeve, for the textile fibres obtainable from its branches and stems. Solvent extracts of the flowers have long been regarded as valuable raw materials in the perfume industry and at Grasse, the French perfumery centre, an average of about 50 tons of petals are treated annually. The odour of broom is powerful and pleasant on the plant but changes very rapidly after picking. With regard to the constituents of broom extracts little is known. A superficial examination of a light petroleum extract of another species of broom, Genista tinctoria, was carried out by Treff, Ritter and Wittrisch (1) who steam-distilled the residue obtained on evaporation of the petroleum and isolated a yellow oil from the distillate. They observed that the oil had a fresh, powerful odour and they recorded its density, optical rotation and other physical properties but did not attempt any true chemical investigation. The first chemical investigation of broom was made by Sabetay and Igolen, who in 1946 published a preliminary note (2) on the constituents of a light petroleum extract of Spartium

junceum. These workers observed that the extract contained free acids (caprylic) which contribute largely to the odour, phenols having a leather or pepper odour, fatty aldehydes, terpenes having a pine oil odour and esters of formic, acetic and higher acids which give the extract a fresh "green" odour. The foregoing publications appear to constitute the total literature on the chemistry of broom and it was therefore felt that a further examination was desirable. The materials for this were procured from a Grasse perfumery where the flowers of Spartium junceum are thoroughly extracted with light petroleum and the solvent removed at low



temperature to give a concentrated oily wax known as the "concrete". The concrete is then extracted with ethanol, the extract chilled to remove any dissolved wax and the solvent evaporated to provide the "absolute" which is

used in compounding the various broom perfumes. The ethanol-exhausted wax, which often retains tenaciously a small amount of odoriferous material, is usually rejected or sold for use in the soap and cosmetic industries. The yield of concrete is about 0.18% of the weight of material extracted and the yield of absolute is about 40% of the weight of concrete. The work reported in this thesis concerns the constituents of the non-saponifiable fractions of wax and absolute.

THEORETICAL.

The examination of the ethanol-exhausted wax was undertaken first, partly because of the comparative abundance of the material and partly to acquire some knowledge of the nature of the substances likely to be encountered in the more valuable absolute, the supply of which was strictly limited.

As a preliminary step the wax, which was in the form of a hard, dark brown cake, was subjected to steam distillation. From the distillate, a small quantity of a brown oil was isolated which possessed a powerful, unpleasant odour. Insufficient material was obtained to permit further investigation. The residual non-volatile wax was now hydrolysed with 7½% ethanolic potassium hydroxide by heating for 7 hours at 100°, the reaction mixture diluted with a large volume of water, transferred to the container of a continuous extraction apparatus (3) and exhausted with ether over a period of several days to extract all non-saponifiable matter. During this time the ether extract in the re-boiler section of the apparatus was withdrawn when necessary and the boiler charged with fresh solvent. The first few extracts, on cooling to room temperature, deposited a buff-coloured waxy solid which was collected as fraction A. A series of

fractions was now obtained which yielded a colourless crystalline material which was collected as fraction B. The later extracts deposited no solid and were combined with the ethereal mother liquors from A and B. By partial evaporation of the solution thus obtained, a buff waxy solid (fraction C) was isolated while complete evaporation furnished fraction D as a brown wax.

In the examination of fraction A, after repeated recrystallisation from various solvents had failed to produce any marked purification the material was dissolved in benzene-light petroleum and filtered through a tower of activated alumina. In this way two fractions were obtained. The first was a colourless solid (88% of weight of A) which had little or no affinity for alumina and crystallised from ethyl acetate as beautiful, soft, lustrous plates of constant m.p. 63°. The substance contained only carbon and hydrogen, was fully saturated and appears to be a long-chain paraffin. This type of compound has been shown to occur to the extent of about 50% in rose wax (4) to about 30% in carnation wax (5) while tobacco leaf wax (6) is made up exclusively of paraffins. From a profound study of these

substances, Chibnall and his colleagues (6) have demonstrated that naturally occurring paraffins are usually mixtures, the components of which contain an odd number of carbon atoms. In this series, microanalysis serves merely to show that carbon and hydrogen are the only elements present and evidence based on crystal spacing data and a standardised melting point determination must be studied before a given sample can be declared a single substance or a mixture. Mixed melting point determinations are also valueless since the paraffins do not depress the melting points of each other. From its m.p. the broom paraffin appears to be n-nonacosane, $C_{29}H_{60}$, (m.p. 63.6°) but lacking X-ray evidence this conclusion must be accepted with reserve.

The second substance from the chromatogram was more firmly retained on the alumina and on purification by recrystallisation from ethyl acetate was obtained as a microcrystalline solid m.p. 76°. The substance was fully saturated, analysed for $C_{24}H_{50}O$, furnished an acetate, $C_{26}H_{52}O_2$, m.p. 60°, and appears to be the long-chain alcohol n-tetracosanol. According to Chibnall (6), however, most of the supposedly pure alcohols from natural sources are mixtures whose

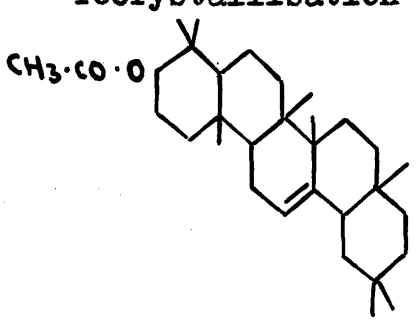
components possess an even number of carbon atoms. The possibility of isolating the individual components of such mixtures in a pure state is considered to be remote.

Fraction B seemed to be homogeneous and on recrystallisation from ethyl acetate was obtained as soft, lustrous, irregular plates m.p. 64 - 65°. The substance was fully saturated, contained only carbon and hydrogen and from a study of tables of melting points of paraffin mixtures compiled by Chibnall, appears to be an approximately equimolar mixture of n-nonacosane, $C_{29}H_{60}$, and n-hentriacontane, $C_{31}H_{64}$.

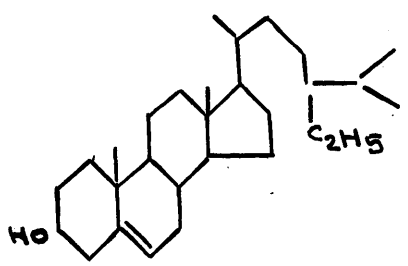
Fraction C, by chromatographic treatment similar to that described for fraction A was separated readily and quantitatively into a paraffin or paraffin mixture m.p. 64° (60%) and a further quantity of n-tetracosanol (40%) identical with that described under fraction A.

Fraction D represented the accumulation of all the most ether-soluble constituents of the non-saponifiable fraction and gave a positive Liebermann-Burchard test. By chromatographic treatment on alumina, using the method of liquid chromatography, the first fractions (i.e. those containing the weakly adsorbed constituents) were

found to be mixtures of paraffins and wax alcohols and were not further examined. Following the above fractions was a series of fractions which gave a pink colouration in the Liebermann-Burchard test. By recrystallisation of the solid recovered from these fractions from ethyl acetate and after removal of a quantity of wax alcohol, concentration of the filtrate yielded an obviously impure material which gave a yellow colour with tetranitromethane and a pink Liebermann-Burchard reaction. Crystallisation of the acetyl derivative of this material from ethanol gave, as least soluble fraction, a crystalline solid m.p. 235° which was shown to be β-amyrin acetate (XXXII) by analysis, optical rotation and mixed m.p. determination with an authentic specimen. Similarly, treatment of the benzoate of the material with ether gave an ether-insoluble residue which when purified by recrystallisation from benzene-acetone analysed for



(XXXII.)



(XXXIII.)

$C_{37}H_{54}O_2$ and had m.p. 232° undepressed when mixed

with β-amyrin benzoate. The other components of the mixture which were probably also triterpenoid in character could not be isolated.

A series of fractions subsequent to those containing β-amyrin provided, in small amount, a solid which when purified by recrystallisation from methanol was obtained as large, colourless plates m.p. 136.5°. The substance gave a blue-black → dark green Liebermann-Burchard colour and provided an acetate, benzoate and 3:5-dinitrobenzoate whose characteristics were in good agreement with those of β-sitosterol (XXXIII) recorded in the literature. The quantity of sitosterol available did not permit an examination for the presence of stigmasterol and sitostanol (7), two components commonly found in sitosterol mixtures. A Tortelli-Jaffe test on a portion of the material recovered from the methanolic mother liquors was negative indicating the absence of the ∝-sitosterol group.

No other pure substances were isolated from the chromatogram.

Since it now seemed unlikely that anything further of interest would be isolated from the wax , the work was discontinued and the investigation of the absolute begun. After preliminary tests had shown that the

absolute contained sulphur and a large amount of free acid, the latter was removed by extraction of an ethereal solution of the absolute with 4% aqueous potassium hydroxide solution and the resulting acid--free product (54% of the absolute) saponified with 5% ethanolic potassium hydroxide. The non-saponifiable fraction (20% of the absolute) thus obtained, was dissolved in benzene, the solution filtered to remove a suspended solid (compound A) and the filtrate chromatographed on alumina (chromatogram 1) by the method of liquid chromatography. The benzene-insoluble compound A, on purification, was obtained as colourless gleaming plates m.p. 97°, which gave no tetranitromethane or Liebermann-Burchard colouration. Compound A was later found to be identical with compound L.

The early fractions from the chromatogram of the non-saponifiable fraction provided a yellow sulphur-containing oil followed by a brown oil which had a few colourless crystals in suspension and possessed a pleasant, terpene-like odour. Neither material has been examined. In a note on the constituents of a light petroleum extract of Spartium junceum, Sabetay and Igolen (2) reported the probable

presence of sulphur but did not isolate any sulphur--containing compound.

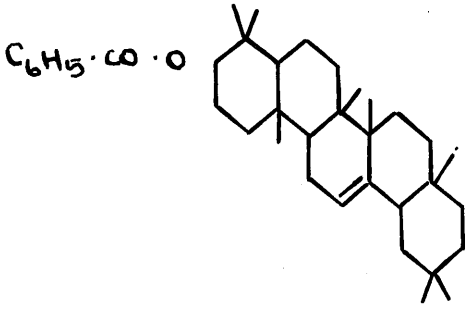
The next main chromatographic fraction, after purification by recrystallisation from ethyl acetate and methanol, gave compound B as a colourless solid m.p. 66°. From microanalyses, molecular weight and active hydrogen determinations, B appears to have the formula $C_{20}H_{41}OH$ or $C_{22}H_{45}OH$. The substance is fully saturated, showed no optical activity, gave no colour in the Liebermann-Burchard test and exhibited no selective absorbtion in the ultra-violet. In attempts to prepare derivatives, B was treated with acetic anhydride, 3:5-dinitrobenzoyl chloride, p-nitrobenzoyl chloride and phenyl isocyanate in turn. In no instance was there an indication of any reaction having occurred. By oxidation of compound B with chromium trioxide in glacial acetic acid solution a product was obtained whose analysis differed little from that of B and which crystallised from ethanol as soft, gleaming plates of constant m.p. 58° undepressed when mixed with compound B. Despite this fact, the great improvement in the crystalline appearance of the oxidation product and the absolute constancy of the m.p. make it difficult to

believe that the product is merely unchanged starting material. Also it seems probable that B is a long-chain aliphatic alcohol and in that case the mixed m.p. evidence could be disregarded. The result of an active hydrogen determination on the oxidation product which will decide definitely whether it is unchanged starting material, was not available in time for inclusion in this thesis. Since B did not yield an acid on chromic acid oxidation under conditions which have been used successfully in the oxidation of long-chain primary alcohols and did not form derivatives with reagents which normally react readily with such alcohols, it seems unlikely that B contains the grouping $-CH_2OH$. If B is a tertiary alcohol, chromic acid oxidation should have resulted in the disruption of the molecule which would have been revealed by a change in analysis of the product. No such change occurred. The remaining possibility is that B is a secondary alcohol. The fact that B displays no optical activity can be disregarded since the rotations of unsymmetrical long--chain secondary alcohols are often so small as to be within the experimental error involved in the determination. The drop of 8° in m.p. on oxidation is quite reasonable

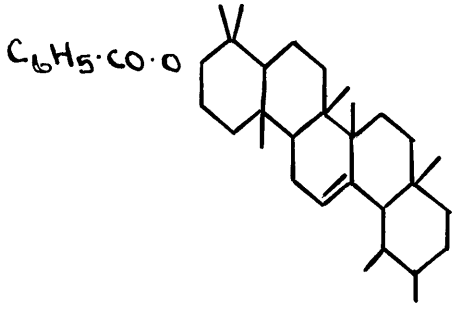
for the conversion of a long-chain secondary alcohol to a ketone. For example, d-2-eicosanol (8), $C_{20}H_{42}O$, m.p. 63°, on chromic acid oxidation gives 2-eicosanone $C_{20}H_{40}O$, m.p. 59°. An attempt to prepare a semicarbazone of the oxidation product of B by methods which provided a quantitative yield with 2-eicosanone, was unsuccessful. A disturbing fact is that d-2-eicosanol forms, with ease, a series of derivatives with the reagents which did not react with B.

The fraction of chromatogram 1 succeeding that containing B afforded a yellow-white solid which gave a pink Liebermann-Burchard colouration and appeared to be a mixture of triterpenes. The mixture was benzoylated by heating with pyridine and benzoyl chloride for 4 hours at 100°, the mixed benzoates dissolved in light petroleum and examined on alumina by the method of liquid chromatography (chromatogram 2). The early fractions consisted mainly of two products, C and D. Compound C, the amount of which was greatly in excess of that of D, separated from benzene-acetone as elongated plates m.p. 235° and was identified as β-amyrin benzoate (XXXIV)(9) by analysis, m.p., mixed m.p. and optical rotation. Further confirmation was obtained by hydrolysis of C,

acetylation of the hydrolysis product and comparison (m.p., mixed m.p. and rotation) of the resulting



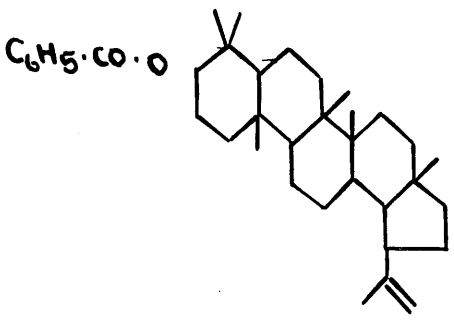
(XXXIV.)



(XXXV.)

acetate with β-amyrin acetate. The identity of compound D as ∝-amyrin benzoate (XXXV)(10) was confirmed by analysis, m.p., mixed m.p. and optical rotation. The next fractions of chromatogram 2, in addition to compounds C and D, contained a more soluble (benzene-acetone) component (E) which crystallised from benzene-acetone as large, colourless, opaque, sponge- or coral-like deposits m.p. 200°. Compound E analysed for $C_{37}H_{54}O_2$, had $[\alpha]_D$ +35°, gave a pale yellow colour with tetranitromethane and a pink Liebermann-Burchard colouration. To obtain an amount large enough to handle, a sample of E in the crude state was hydrolysed with 4% ethanolic potassium hydroxide, the product acetylated and the crude acetate chromatographed on alumina to give two apparently homogeneous substances. The first (less

strongly adsorbed) analysed for $C_{32}H_{52}O_2$, crystallised from chloroform-methanol as colourless plates m.p. 203 - - 205°, $[\alpha]_D$-28.5° and did not depress the m.p. of a sample of acetate prepared from a small, pure specimen of E. This would suggest that the acetate corresponds to E although the change in optical rotation (+35° to -28.5°) is greater than would normally be expected in passing from triterpene benzoate to triterpene acetate. No compound has been found in the literature whose characteristics correspond to those of E. The second and more strongly adsorbed substance, probably $C_{32}H_{52}O_2$, crystallised from chloroform-methanol as felted needles m.p. 165°, $[\alpha]_D$+15.9°, depressed the m.p. of the acetate of compound E and has not been identified. The fractions from chromatogram 2 subsequent to those containing compounds C, D and E, yielded a higher melting material F which crystallised from benzene-acetone in plates m.p. 265° and was shown by analysis, m.p., mixed m.p. and rotation, and by m.p., mixed m.p. and rotation of the acetate (hydrolysis of F and acetylation) to be lupeol



(XXXVI.)

benzoate (XXXVI)(11). The final fractions of chromatogram 2, after purification from acetone, furnished an unidentified compound (G) as a colourless, poorly crystalline solid m.p. 65 - 69° which was fully saturated and gave no Liebermann-Burchard colour. Insufficient material was isolated to allow further purification and examination.

Chromatogram 1, after elution of the triterpene fraction, provided a waxy solid from which, by crystallisation from methanol, three products were obtained. The first (compound H) and least soluble, separated from methanol as small needles or prisms m.p. 71 - 73°, analysed for $C_{24}H_{50}O$ or $C_{26}H_{54}O$, was fully saturated, optically inactive and gave no Liebermann-Burchard colour. Compound H is probably a long-chain aliphatic alcohol but time did not permit the experiments required to substantiate this belief. The second component (I) which also analysed for $C_{24}H_{50}O$ or $C_{26}H_{54}O$, crystallised from methanol as pearly spheres of needles m.p. 56 - 57°, was optically inactive, gave a very weak tetranitromethane colour and a beautiful blue Liebermann-Burchard colour, and furnished a 3:5-dinitrobenzoate m.p. 73 - 74°. It was thought that the blue Liebermann might have been due

to a trace of impurity so, as a test of homogeneity, compound I was chromatographed on alumina. No evidence of the presence of any impurity was obtained and the identity of the substance remains unknown. A Liebermann-Burchard colour usually suggests a triterpene or steroid but the low m.p. and lack of optical activity of I practically exclude this possibility although some sort of cyclic arrangement might be possible. Since the substance is known to possess a primary or secondary hydroxyl group, it had been intended to form a series of derivatives and to carry out a chromic acid oxidation of compound I but pressure of work bearing higher priority prevented this. The third compound separated from methanol as fine needles m.p. 166° which, on acetylation and purification of the acetate, gave β-amyrin acetate as needles m.p. 231° undepressed when mixed with an authentic specimen.

The next fractions from chromatogram 1 furnished a colourless solid which gave a colour sequence of pink ⟶ purple ⟶ dark blue ⟶ green in the Liebermann-Burchard test. After purification by crystallisation from methanol, compound J was obtained as lustrous, elongated plates m.p. 137°, identical (m.p., mixed m.p. and rotation) with the β-sitosterol of broom wax (p.65a).

While recognising the possibility of compound J being a mixture, no attempt was made to examine it for the presence of stigmasterol or sitostanol or to confirm its homogeneity by the oxidative method of Barton and Jones (12).

The succeeding fraction of chromatogram 1 now furnished a brown liquid which contained some solid in suspension. On recrystallisation from methanol, chloroform and repeatedly from benzene, compound K was obtained as microprisms m.p. 104.5°. Compound K, $C_{26}H_{54}O_2$, was fully saturated, gave no Liebermann-Burchard colouration and in view of its great affinity for alumina and its close proximity on the tower to compound L, is probably the straight-chain hexacosane-1:26-diol (XXXVII)

$$HOCH_2-(CH_2)_{24}-CH_2OH$$

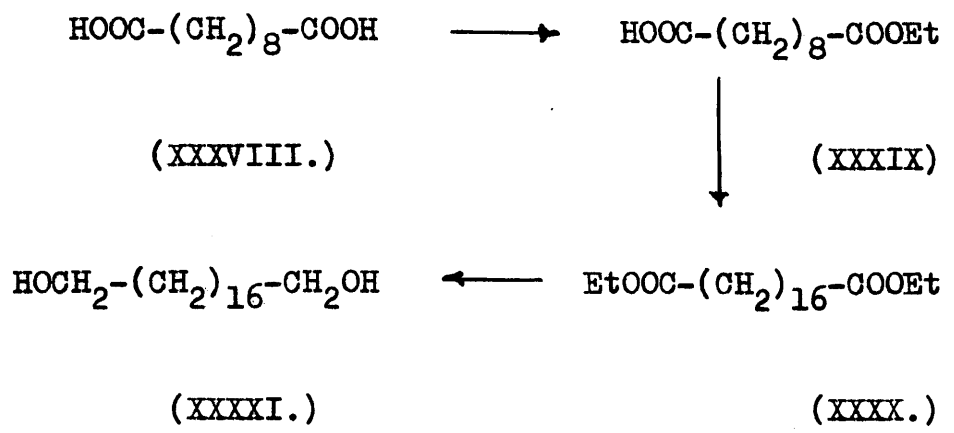
(XXXVII.)

which has not been described. The fact that it preceeded compound L (cf. p. 52) on the tower fits with this conclusion since the two hydroxyl groups with a 26-carbon chain would have less affinity for alumina than with the 18-carbon chain of compound L. An attempted

acetylation of K using acetic anhydride at 100° was unsuccessful and scarcity of material prevented further experimentation.

The final fraction of chromatogram 1, on recrystallisation from methanol and repeatedly from benzene, yielded compound L, $C_{18}H_{38}O_2$, as soft gleaming plates m.p. 99°. By reason of its strong affinity for alumina, its complete saturation, its possession of two active hydrogen atoms, its lack of absorbtion in the ultra-violet and its negative Liebermann-Burchard reaction, compound L was thought to be an octadecanediol. Since it is an established fact that among the naturally occurring aliphatic acids, alcohols etc., straight-chain substances predominate and since numerous straight-chain substances are known, as for example, the ω-hydroxy acids sabinic (12-hydroxylauric) and juniperic (16-hydroxypalmitic)(13) and the α:ω-dibasic acids of Japan wax (14), whose terminal carbon atoms carry a functional group, it seemed probable that L was octadecane-1:18-diol (XXXXI) m.p. 99°. This conclusion was borne out by the synthetic preparation of octadecane-1:18-diol, its diacetate, di-3:5-dinitro--benzoate, dibenzoate and octadecane-1:18-dioic acid (by chromic acid oxidation of the diol). In each case the product was identical with the corresponding derivative of compound L. The specimen of

octadecane-1:18-diol (15) was prepared by the route indicated in which sebacic acid (XXXVIII) was half



esterified to give ethyl hydrogen sebacate (XXXIX) which was electrolysed in absolute methanol solution by the general method described by Greaves, Linstead, Shephard, Thomas and Weedon (16) to give diethyl octadecane-1:18--dioate (XXXX) in 60% yield. The conversion of the diester to the diol (XXXXI) was accomplished in 88% yield using lithium aluminium hydride (17), the previous best yield reported (18) being 66% by the Bouveault-Blanc method.

The isolation of octadecane-1:18-diol from Spartium junceum appears to be unique. No account of the isolation of a substance of this nature from a plant source ~~appears~~

appears in the literature and it is interesting to speculate on the possible mechanism of formation of the diol in the plant. As an explanation of the formation of the $\alpha:\omega$-hydroxy acids in plants, Chibnall (19) has suggested that they arise by ω-oxidation of normal fatty acids. For example, palmitic acid (XXXXII)

$$CH_3-(CH_2)_{14}-COOH \rightarrow HOOC-(CH_2)_{14}-COOH \rightarrow HOCH_2-(CH_2)_{14}-COOH$$

(XXXXII.) (XXXXIII.) (XXXXIV.)

on ω-oxidation would give thapsic acid (XXXXIII) which on partial reduction would give juniperic acid (XXXXIV). If the remaining carboxyl group were similarly reduced the product would be an $\alpha:\omega$-diol. The analogous

$$CH_3-(CH_2)_{16}-COOH \rightarrow HOOC-(CH_2)_{16}-COOH \rightarrow HOCH_2-(CH_2)_{16}-COOH$$

(XXXXV.) (XXXXVI.) $\downarrow$ (XXXXVII.)

$$HOCH_2-(CH_2)_{16}-CH_2OH$$

sequence for the formation of octadecane-1:18-diol would have stearic acid (XXXXV) as starting material and it would be of considerable interest if the intermediate octadecane-1:18-dioic acid (XXXXVI) and the $\alpha:\omega$-hydroxy

acid (XXXXVII) could be isolated from the acids of Spanish broom.

The identification of octadecane-1:18-diol concluded the examination of the non-saponifiable fraction of broom absolute.

EXPERIMENTAL.

Broom Wax.

The ethanol-exhausted wax was obtained as a hard, dark brown cake which possessed a powerful, rather sharp odour.

Removal of Volatile Components.- A mixture of broom wax (500g.) and water (300 - 400 c.c.) was maintained at a temperature of 90 - 100° while steam was introduced at atmospheric pressure. The distillate (1.5 l.) which contained no water-immiscible material and which had a powerful, unpleasant odour, was exhausted with ether in a continuous extraction apparatus. Evaporation of the solvent from the extract through a small fractionating column gave, as residue, a negligible amount of a brown oil which was discarded. The residual non-volatile wax from the steam distillation when cool, solidified to a firm, dark brown block which was hydrolysed as described below.

Hydrolysis of Wax.- A solution of the foregoing non-volatile wax (97g.) in ethanolic potassium hydroxide (1156 c.c. of 7.5%) was refluxed on a steam bath for 7 hours. The contents of the reaction flask

were then transferred to the container of a continuous ether extractor, diluted to ca. 5 litres with water and the resulting thick, soapy suspension extracted with ether over a prolonged period. The concentrated ethereal extract in the boiler was withdrawn when necessary (i.e. when the hot solution became saturated) and fresh ether (500 c.c.) introduced. In the course of the extraction the following series was collected.

Extract 1. The yellow ether solution, on cooling, deposited a waxy, buff-coloured solid (4.0g.) m.p. 58°.

Extract 2. This was similar to 1 and provided a solid (12.0g.) m.p. 59°.

Extract 3. This yielded a solid (8.5g.) m.p. 58 - 62°.

Extract 4. This was almost colourless and deposited a crystalline solid (3.4g.) as small irregular plates m.p. 64°.

Extract 5. The extract was colourless and deposited a crystalline solid (5.2g.) m.p. 64°.

Extract 6. This yielded a colourless, crystalline product (7.6g.) m.p. 64°.

Extract 7. This gave a colourless, crystalline solid (3.2g.) m.p. 65°.

From extract 7 onwards no solid was deposited when the extract was cooled. Extraction was continued until

the amount of material in the extract was negligible. The solids from extracts 1 - 3 were combined (A; 24.5g.) as were those from extracts 4 - 7 (B; 19.4g.). The mother liquors from extracts 1 - 3 were combined, the ether (containing some alcohol extracted from the hydrolysis mixture) removed under slight vacuum and the residue dissolved in fresh ether (1 l.). This solution was added to the mother liquors from extracts 4 - 7 together with the later extracts which had not deposited solid and the whole (4 l.) washed thoroughly with water and dried (sodium sulphate). The solution was now concentrated to ca. 500 c.c., cooled, and the deposited solid collected (C; 10g., m.p. 55 - 63°). Evaporation of the ether from the filtrate yielded a brown, waxy solid (D; 12.8g.).

Examination of Fractions A - D.- Fraction A. A portion (2.5g.) of the solid was dissolved in benzene-light petroleum (b.p. 60 - 80°)(300 c.c., 2:1) and the pale yellow solution filtered through a tower (20 x 3 cm.) of activated alumina (Brockmann II). On developement of the chromatogram with the same solvent mixture, the whole of the solute was recovered as two fractions. The first fraction (2.1g.; 1 litre of eluate) yielded a

colourless solid which had no affinity for alumina and which on recrystallisation from ethyl acetate was obtained as lustrous plates of constant m.p. 63°.

Found: C, 85.4; H, 14.6.

Calc. for $C_{29}H_{60}$: C, 85.3; H, 14.7%.

The second fraction (0.3g.; 1.4 litres of eluate) was more firmly adsorbed on alumina and after three crystallisations from ethyl acetate yielded a colourless, microcrystalline solid m.p. 75 - 76°.

Found: C, 81.3; H, 14.0.

Calc. for $C_{24}H_{50}O$: C, 81.4; H, 14.1%.

The acetyl derivative, prepared by refluxing the material with acetic anhydride, crystallised from light petroleum (b.p. 40 - 60°) as soft, colourless plates m.p. 59 - 60°.

Found: C, 79.0; H, 13.2.

Calc. for $C_{26}H_{52}O_2$: C, 78.8; H, 13.1%.

Fraction B. The substance was recrystallised from ethyl acetate from which it separated as colourless, soft, irregular plates of constant m.p. 64 - 65°.

Found: C, 85.5; H, 14.9.

Calc. for $C_{29}H_{60}$: C, 85.3; H, 14.7.

$C_{31}H_{64}$: C, 85.3; H, 14.7%.

Fraction C. By methods identical with those described under fraction A, fraction C was resolved into a hydrocarbon (60%) which crystallised from ethyl acetate as soft, colourless plates m.p. 64° undepressed when mixed with the paraffin m.p. 63° from A or with that m.p. 65° from fraction B, and an alcohol m.p. 76° (40%) identical with that described under A.

Fraction D. A quantity (12.1g.) of the brown wax was dissolved in benzene-light petroleum (b.p. 40 - 60°) (500 c.c., 1:1) and passed down a tower (20 x 3 cm.) of alumina (grade II). The chromatogram was eluted with benzene- light petroleum (1:1) to give the fractions indicated in table 2.

Preliminary crystallisations of fraction 1 showed that the solid was a mixture of paraffins and alcohols whose resolution was not attempted.

Fraction 2 was dissolved in boiling ethyl acetate (30 c.c.) from which, on cooling, a colourless solid separated as small, radiating clusters of rods or needles. These were collected (1.2g.; filtrate A) and after repeated recrystallisation from ethyl acetate had m.p. 74° undepressed when mixed with the alcohol m.p. 76°

Table 2.

Fraction.	Solvent.	Wt. of Fraction.	Description.
1	Benzene-petrol (1:1; 1.3 l.)	5.7g.	Brown wax m.p. 63°.
2	Benzene-petrol (1:1; 2.2 l.)	3.4g.	Solid m.p. ca. 140°. Pink Lieb.
3	Benzene-petrol (1:1; 1 l.)	0.5g.	Oily solid. Lieb.: blue dark green.
4	-	0.8g.	Solid m.p. ca. 130°. Lieb.: blue dark green.
5	-	0.6g.	Resin.
6	-	0.4g.	Brittle resin.

Fractions 4 - 6 were obtained by dividing the column mechanically into 3 sections. Each section was eluted with benzene-ethanol (100 c.c., 9:1), the suspension filtered and the alumina washed on the filter with a further quantity (50 c.c.) of the same mixture.

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described under fraction A. The substance formed an acetate m.p. 59° undepressed on admixture with the acetate (m.p. 60°) of the foregoing alcohol. Filtrate A, on concentration, yielded three successive crystalline crops m.p. 155° (0.6g.), m.p. 163° (0.3g.) and m.p. 147° (0.8g.) respectively.

Isolation of β-Amyrin.- (a) As the acetate. A portion (0.14g.) of the foregoing material m.p. 163° was refluxed with acetic anhydride (3 c.c.) for 3 hours, the solution cooled and poured into water. The slightly discoloured precipitate thus obtained was collected, washed with water, dried and treated with boiling ethanol (5 c.c.). The suspension was filtered hot and the residue recrystallised once from glacial acetic acid and then from ethanol from which it separated as long colourless needles m.p. 235°, $[\alpha]_D$ +78.2° (c, 1.54 in benzene). The m.p. of the substance was not depressed when a specimen was mixed with authentic β-amyrin acetate (m.p. 236°) of specific rotation +78.6°.

Found: C, 81.9; H, 11.4.

Calc. for $C_{32}H_{52}O_2$: C, 82.1; H, 11.1%.

The cooled ethanolic filtrate from the above hot filtration deposited a crop of colourless elongated

plates m.p. 225° and when concentrated to small bulk yielded a further crop m.p. 215°. In each case the m.p. was raised when the substance was mixed with authentic β-amyrin acetate.

(b) As the benzoate. A mixture of the material m.p. 147° (0.8g.), pyridine and benzoyl chloride was heated for 5 hours on a steam bath, the dark red reaction mixture dissolved in benzene (50 c.c.) and the solution washed with 3N hydrochloric acid and repeatedly with sodium hydroxide (5%). Since the odour of benzoyl chloride still persisted the benzene was removed under vacuum and the residual gum shaken with dilute sodium hydroxide (2%) for several hours after which it was dissolved in benzene (50 c.c.) washed with salt water and dried (sodium sulphate). The sticky residue obtained by evaporation of the benzene was dissolved in boiling ethanol to give, on cooling, a colourless crystalline solid (0.3g.) m.p. 185°. This material was shaken with ether (2 c.c.) at room temperature, the suspension filtered and the residue (0.08g.) recrystallised twice from benzene-acetone from which it separated as colourless gleaming plates m.p. 232° undepressed when mixed with authentic β-amyrin benzoate (m.p. 233°).

Found: C, 83.1; H, 10.1.

Calc. for $C_{37}H_{54}O_2$: C, 83.8; H, 10.2%.

The ether filtrate was evaporated to dryness to give a pale yellow resin (0.2g.) which on crystallisation from benzene-ethanol provided a crop of crystals m.p. 190°, raised to 193° when mixed with β-amyrin benzoate and depressed to 172° when mixed with α-amyrin benzoate. Two further crops were obtained from the mother liquor which had m.p. 187° and 180° respectively. In each case the melting point was raised when the substance was mixed with β-amyrin benzoate and depressed when mixed with α-amyrin benzoate.

Fraction 3 of the chromatogram of fraction D has not been examined.

Isolation of β-Sitosterol.- Fraction 4 of the chromatogram provided a yellow-brown solid (0.81g.) which was recrystallised repeatedly from methanol to give β-sitosterol as lustrous irregular plates of constant m.p. 136.5°, $[\alpha]_D$-34.4° (c, 0.61 in chloroform). In the Liebermann-Burchard test the colour sequence was dark purple ⟶ blue-green.

β-Sitosterol Acetate. A portion (0.09g.) of the foregoing sterol was heated for 4 hours on a steam bath

with acetic anhydride (2 c.c.), the solution poured into water and the aqueous suspension extracted with ether. The extract was washed with sodium carbonate solution (5%) and water, dried (sodium sulphate) and evaporated to dryness to give β-sitosterol acetate as a slightly discoloured solid which after two crystall--isations from ethanol was obtained as colourless prisms m.p. 127.5°, $[\alpha]_D$ -37.5° (c, 1.02 in chloroform).

β-Sitosterol Benzoate. The sterol (0.10g.) was dissolved in pyridine (1.5 c.c.) and treated with benzoyl chloride (0.03 c.c.), the mixture being heated on a steam bath for 1 hour. At the end of this time the mixture was diluted with ether and the ethereal solution washed successively with 3N hydrochloric acid, 2% sodium hydroxide and water. Removal of the ether from the dried (sodium sulphate) solution gave the benzoate as a colourless solid which after purification from benzene-ethanol was obtained as small leaflets m.p. 143 - 144.5°, $[\alpha]_D$ -14.4° (c, 1.11 in chloroform).

β-Sitosterol 3:5-Dinitrobenzoate. A mixture of the sitosterol (0.2g.), dry pyridine (2 c.c.) and 3:5-dinitrobenzoyl chloride (0.13g.) was heated at 100° for 1 hour, diluted with ether (50 c.c.) and the solution

washed successively with 3N hydrochloric acid, sodium hydroxide (2%) and water. The dried (sodium sulphate) solution, on evaporation furnished a cream-coloured solid which was recrystallised several times from ethyl acetate-ethanol to give β-sitosterol 3:5-dinitrobenzoate as pale yellow plates m.p. 198 - 200°, $[\alpha]_D$ -10.1° (c, 0.50 in chloroform).

A comparison of the properties of the sitosterol from broom wax with those recorded for β-sitosterol is given in table 3.

Fractions 5 and 6 of the chromatogram were not examined.

Broom Absolute.

The absolute was supplied as a brown semi-solid material which possessed a pleasant, sweet odour, reminiscent of raisins, intermingled with a rancid fatty odour. An elements test revealed the presence of sulphur and the absence of nitrogen and halogen, while with litmus a strongly acid reaction was obtained.

Table 3.

Sterol.		Acetate.		Benzoate.		3:5-Dinitrobenzoate.	
M.p.	$[\alpha]_D$	M.p.	$[\alpha]_D$	M.p.	$[\alpha]_D$	M.p.	$[\alpha]_D$
136.5°	-34.4°	127.5°	-37.5°	144.5°	-14.4°	200°	-10.1°[1]
138.5°	-34.0°	127.5°	-	146.5°	-	209°[2]	-
137°	-36.6°	126°	-41.0°	147°	-13.8°	203°	-10.4°[3]
135.5°	-34.2°	127°	-34.7°	146°	-14.2°	209°	-21.7°[4]
137°	-31.5°	123°	-36.7°	147°[5]	-	-	-

1 Values for broom wax sitosterol.

2 β-Sitosterol of Cook and Paige (7).

3 Wallis and Chakravorty (20).

4 Simpson and Williams (21).

5 Ichiba (22).

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Removal of Free Acids.- A solution of the absolute (194g.) in ether (1200 c.c.) was extracted with potassium hydroxide solution (4%; 5 x 200 c.c.) until free from acid, washed with water and dried (sodium sulphate). The potassium hydroxide extract was extracted with ether (5 x 200 c.c.), the ether extract washed with water, dried (sodium sulphate) and combined with the foregoing ethereal solution. Evaporation of the ether under slightly diminished pressure gave a fairly mobile brown oil (105g.) with a rich raisin-like odour. The foregoing potassium hydroxide extract (containing the "free" acids as K salts) was set aside for subsequent examination.

Saponification of the Neutral Fraction.- The neutral fraction (105g.), obtained as described above, was refluxed for 1 hour with ethanolic potassium hydroxide (5%; 1 l.). The greater part of the ethanol was then removed by distillation under slight vacuum, water being added at intervals to maintain a constant volume. When ca. 1 litre of distillate had collected, the hydrolysis mixture, which was chocolate-brown in colour and possessed a pleasant geraniol-like odour, was

diluted with water (1 l.) and extracted repeatedly with ether (15 x 200 c.c.), the extract washed with water, dried (sodium sulphate) and evaporated to dryness to give the unsaponifiable matter (40g.) as a clear, wine-red viscous liquid.

Chromatogram 1. Chromatography of Non-Saponifiable Fraction.- The non-saponifiable fraction (40g.) was dissolved in dry benzene (1 l.), the amber solution filtered to remove a suspended solid (0.38g.; compound A) and passed down a tower (35 x 6 cm.) of activated alumina (Brockmann II). The chromatogram was developed with benzene and benzene-ethanol to give the fractions shown in table 4.

Examination of Compound A and Chromatogram Fractions.- Compound A: The substance, after seven crystallisations from ethyl acetate was obtained as soft, gleaming plates m.p. 97° which gave no tetranitromethane or Liebermann--Burchard colourations. The m.p. was undepressed when the substance was mixed with octadecane-1:18-diol (compound L).

Table 4.

Fraction.	Solvent.	Wt. of Fraction.	Description.
1	Benzene (2 l.)	4.46g.	Yellow oil.
2	Benzene (0.6 l.)	0.09g.	Brown gum plus a few crystals. Pleasant odour.
3	Benzene (1.2 l.)	0.07g.	Brown gum.
4	Benzene (6.8 l.)	2.63g.	Brown oily wax.
5	Benzene (1.2 l.) Benzene-ethanol (99.5:0.5; 8.6 l.)	10.39g.	Yellow-white solid. Pink Liebermann.
6	Benzene-ethanol (99.5:0.5; 8.0 l.)	8.13g.	Low-melting wax. Purple Liebermann.
7	Benzene-ethanol (99.5:0.5; 4.0 l.)	1.33g.	Solid m.p. above 100° Lieb. purple blue-black green.
8	-	2.75g.	As for fraction 7.
9	-	1.86g.	Brown oil plus trace of solid.
10	-	1.14g.	Brown oil.
11	-	1.00g.	Brown oil plus solid.
12	-	1.60g.	Brown oily solid.

Table 4.(contd.)

13	-	0.50g.	Viscous brown liquid.

Fractions 8 - 13 were obtained by the following procedure. The chromatogram was divided mechanically into 16 equal sections. Each section was eluted with benzene-ethanol (3:1; 200 c.c.) the alumina collected and washed on the filter with a further quantity of benzene-ethanol (10:1; 55 c.c.). Of the fractions obtained on evaporation of the solvent, the first two were combined as fraction 8, the next five as fraction 9, the following four as fraction 10, the next two as fraction 11, the succeeding two as fraction 12 and the remaining fraction was taken as 13.

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Fraction 1: A sodium fusion indicated the presence of sulphur. No further examination was made.

Fractions 2 and 3 were not examined.

Fraction 4: Isolation of an Unidentified Compound (B).- The yellow-brown waxy solid (2.63g.) was recrystallised once from ethyl acetate and thrice from methanol to give compound B (0.6g.) as colourless, circular clusters of soft needles or rods of constant m.p. 65 - 66°.

Found:	C,81.7; H,13.8.	Active H: 0.36%.	M.W. 318.	
	C,81.4; H,13.5.			
Calc. for $C_{20}H_{42}O$:	C,80.5; H,14.1.	Active H: 0.34%	M.W. 298.	
$C_{22}H_{46}O$:	C,80.9; H,14.1.	0.31%	M.W. 326.	
$C_{24}H_{50}O$:	C,81.4; H,14.1%.	0.29%	M.W. 354.	

Compound B does not contain sulphur, nitrogen or halogen, gives no tetranitromethane or Liebermann-Burchard colouration and shows no selective absorbtion in the ultra-violet.

Attempted Acetylation of B. A mixture of compound B (100 mg.) and acetic anhydride (2 c.c.) was refluxed for 2 hours, the clear solution poured into water and

the liquid mixture extracted with ether (3 x 15 c.c.). Evaporation of the dried (sodium sulphate) extract gave a colourless oil (105 mg.) which could not be induced to crystallise at room temperature.

Attempted Preparation of a 3:5-Dinitrobenzoate of B. A mixture of compound B (50 mg.), pyridine (2 c.c.) and 3:5-dinitrobenzoyl chloride (30 mg.) was heated at 100° for 2 hours, the reaction mixture diluted with ether (50 c.c.) and the solution washed successively with 3N hydrochloric acid, 5% sodium carbonate and water. The residue obtained on evaporation of the ether, on recrystallisation from methanol separated as small, poorly defined crystals m.p. 63 - 66° undepressed when mixed with compound B.

Attempted Preparation of a ρ-Nitrobenzoate of B. A mixture of compound B (40 mg.), ρ-nitrobenzoyl chloride (25 mg.) and pyridine (1 c.c.) was heated at 100° for 1 hour, diluted with ether and the ether solution washed with hydrochloric acid, sodium carbonate and water in turn. Evaporation of the solvent gave a yellow oil which refused to crystallise.

Attempted Preparation of a Phenylurethane of B. A mixture of compound B (80 mg.), phenyl isocyanate (40 mg.) and dry petrol (5 c.c.; b.p. 100 - 120°) was heated at 100° for 3 hours and then filtered hot into a small conical flask. When crystallisation did not occur, the solvent was removed and the residue recrystallised from methanol from which it separated as circular clusters of needles or rods m.p. 56 - 62° undepressed when mixed with compound B. An elements test showed that the substance did not contain nitrogen.

Attempted Oxidation of B. To a warm solution of compound B (150 mg.) in stabilised glacial acetic acid (4 c.c.) was added gradually a solution of chromium trioxide (133 mg.) in glacial acetic acid (4 c.c.) and the mixture allowed to stand at room temperature for 30 minutes. It was then poured into water, the precipitate extracted with ether (4 x 25 c.c.), the extract washed with water (4 x 50 c.c.) and dried (sodium sulphate). The waxy solid obtained on evaporation of the ether was treated with hot sodium carbonate solution (2%; 50 c.c.), the cooled suspension extracted with ether (4 x 15 c.c.), the extract dried (sodium sulphate) and evaporated to give a colourless solid (100 mg.) which was crystallised

four times from methanol and finally from ethanol from which it separated as soft gleaming plates of constant m.p. 58° raised to 59° when mixed with compound B.

Found: C, 81.5; H, 14.1.

Calc. for $C_{20}H_{40}O$: C, 81.1; H, 13.5.

$C_{22}H_{44}O$: C, 81.5; H, 13.6%.

The foregoing ether-extracted sodium carbonate solution was acidified and again extracted with ether. Evaporation of the solvent gave a waxy solid (35 mg.) which could not be redissolved in sodium carbonate and which on recrystallisation from ethanol was obtained as lustrous plates m.p. 58° alone and when mixed with the substance previously isolated from the oxidation.

Attempted Preparation of a Semicarbazone of the Oxidation Product. A mixture of the supposed ketone from the foregoing oxidation (20 mg.), semicarbazide hydrochloride (16 mg.), sodium acetate (16 mg.) and aqueous ethanol (80%; 2 c.c.) was heated at 50 - 60° for 10 minutes, cooled and the crystalline solid collected. Recrystallisation from ethanol gave the product as colourless, lustrous plates m.p. 58° undepressed when mixed with compound B.

Fraction 5: Benzoylation.- A mixture of the solid (10g.), dry pyridine (15 c.c.) and benzoyl chloride (3.5 c.c.) was heated for 4 hours at 100°, diluted with benzene (300 c.c.) and the resulting solution washed successively with 3N hydrochloric acid (4 x 20 c.c.), water, potassium hydroxide (3%; 4 x 20 c.c.) and finally with a large volume of water. Evaporation of the solvent from the dried (sodium sulphate) solution furnished a brown resin (12g.) which gradually solidified on standing.

Chromatogram 2. Chromatographic Separation of Benzoates.- The benzoates (12g.), obtained as described above, were dissolved in light petroleum (300 c.c.; b.p. 40 - 60°) to give a lemon-yellow solution which was filtered to remove a small amount of suspended matter and run through a tower (30 x 5 cm.) of activated alumina (Brockmann II) to give the fractions shown in table 5.

A benzene-acetone solution of fraction I, on slow cooling, afforded two crystalline products. The less soluble product (C) crystallised as elongated plates m.p. 234° (ca. 200 mg.) and the other (D)

Table 5.

Fraction.	Solvent.	Wt. of Fraction.	Description.
I	Benzene-petrol (5:95; 0.5 l.)	0.90g.	Colourless solid m.p. ca. 190°. Pink Liebermann.
II	Benzene-petrol (5:95; 1.0 l.)	1.58g.	As for I.
III	Benzene-petrol (5:95; 0.7 l.)	0.71g.	Brittle solid m.p. ca. 175°. Pink Liebermann.
IV	Benzene-petrol (5:95; 1.3 l.)	1.26g.	Brittle solid m.p. ca. 150°. Pink Liebermann.
V	Benzene-petrol (10:90; 0.8 l.)	0.81g.	Brittle solid m.p. ca. 190°. Pink Liebermann.
VI	Benzene-petrol (15:85; 0.4 l.) (15:85; 0.8 l.)	2.28g.	Brittle solid m.p. ca. 220°. Pink Liebermann.
VII	Benzene-petrol (15:85; 0.4 l.)	0.29g.	Brittle solid m.p. ca. 210°. Pink Liebermann.
VIII	Benzene-petrol (1:1; 0.4 l.) Benzene (0.4 l.)	0.40g.	Resin. Pink Lieb.
IX	-	0.10g.	Yellow gum. No Lieb.
X	-	0.07g.	Oily solid. No Lieb.
XI	-	1.11g.	Yellow, oily wax. No Liebermann.

Table 5. (contd.)

XII	-	0.33g.	Yellow resin. No Liebermann.
XIII	-	1.18g.	Mobile brown oil. No Liebermann.

"Petrol" in the above table refers to light petroleum b.p. 40 - 60°.

Fractions IX - XIII were obtained by dividing the chromatogram into 10 equal sections. Each section was eluted with benzene-ethanol (4:1; 125 c.c.) filtered, and the alumina washed on the filter with a further quantity of benzene-ethanol (1:1; 50 c.c.). The material obtained on evaporation of the solvent was combined as follows. The first section gave fraction IX, the next, fraction X, the following six sections gave fraction XI, and the remaining sections gave fractions XII and XIII respectively.

separated as large, dense, transparent prisms m.p. 198° (ca. 60 mg.).

Fraction II, on similar treatment, yielded further quantities of compounds C (ca. 400 mg.) and D (ca. 60 mg.) and in addition, the mother liquors provided a third product (E) as opaque, sponge-like deposits m.p. 200° (ca. 25 mg.).

Fraction III furnished further quantities of compounds C (90 mg.) and E (ca. 300 mg., crude).

Fraction IV consisted mainly of compound E (ca. 900 mg., crude).

No homogeneous product was isolated from fraction V.

Fraction VI provided compound F (ca. 100 mg.) as colourless, elongated plates m.p. 265°.

No homogeneous product was isolated from fraction VII.

Fractions VIII - X were not examined.

Fraction XI, on recrystallisation from acetone gave compound G as a colourless solid m.p. 69°.

Fractions XII and XIII were not examined.

Examination of Compounds C,D,E,F and G.- Compound C: Identification as β-amyrin benzoate. The substance crystallised from benzene-acetone as large, gleaming,

rectangular plates m.p. 235° undepressed when mixed with authentic β-amyrin benzoate.

Found: C, 83.8; H, 10.4.

Calc. for $C_{37}H_{54}O_2$: C, 83.7; H, 10.3%.

$[\alpha]_D$ +96.6° (c, 1.17 in chloroform).

lit. value (23) $[\alpha]_D$ +100°.

The substance gave a pale yellow colouration with tetranitromethane and a pink Liebermann-Burchard test.

A portion of the benzoate was hydrolysed with ethanolic potassium hydroxide (4%; 4 hours at 100°) and the product, without further purification, acetylated by means of pyridine and acetic anhydride (1½ hours at 100°). The acetate thus obtained crystallised from chloroform-methanol as well formed needles m.p. 233° undepressed when mixed with authentic β-amyrin acetate (m.p. 235°).

$[\alpha]_D$ +79.3° (c, 1.27 in chloroform).

lit. value (23) $[\alpha]_D$ +78.6°.

Compound D: Identification as α-amyrin benzoate.

The compound, which gave a yellow tetranitromethane colour and a pink Liebermann-Burchard colouration, separated from benzene-acetone as large, transparent

prisms m.p. 198° undepressed when mixed with authentic ∝-amyrin benzoate (m.p. 198°).

Found: C, 83.3; H, 10.0.

Calc. for $C_{37}H_{54}O_2$: C, 83.7; H, 10.3%.

$[\alpha]_D$ +91.8° (c, 1.06 in chloroform).

lit. value (23) $[\alpha]_D$ +94.6°.

Compound E: The substance, which gave a yellow tetranitromethane colour and a pink Liebermann, was obtained from benzene-acetone as characteristic, large sponge-like formations m.p. 200°, depressed strongly when mixed with ∝-amyrin benzoate and slightly with β-amyrin benzoate.

Found: C, 83.3; H, 9.9.

Calc. for $C_{37}H_{54}O_2$: C, 83.7; H, 10.3%.

$[\alpha]_D$ +35.4° (c, 0.99 in chloroform).

A quantity of the benzoate (1.25g.; crude, m.p. 184°) was hydrolysed by refluxing for 10 hours with ethanolic potassium hydroxide (4%; 50 c.c.) to give a slightly discoloured solid (1.0g.) m.p. 168° which was acetylated using pyridine (5 c.c.) and acetic anhydride (10 c.c.) (6 hours at 100°). The product was a colourless solid (1.05g.) m.p. 170 - 200° which was recrystallised from

chloroform-methanol. After removal of a first crop (382 mg.) of β-amyrin acetate the mother liquor was evaporated to dryness, the residue dissolved in light petroleum (b.p. 60 - 80°) and chromatographed on alumina. Three main fractions were collected. The first (112 mg.) which was least strongly adsorbed, had m.p. 194 - 205° and was obviously a mixture. The intermediate fraction (265 mg.) after three crystallisations from chloroform--methanol separated as gleaming hexagonal plates m.p. 203 - 205° which lost their lustre and became opaque on exposure to the atmosphere.

Found: C, 81.9; H, 10.9.

Calc. for $C_{32}H_{52}O_2$: C, 82.1; H, 11.1%.

$[\alpha]_D$ -28.5° (c, 0.77 in chloroform).

The substance gave a pale yellow tetranitromethane colour while the sequence in the Liebermann-Burchard test was salmon-pink ⟶ blood red.

The final fraction (189 mg.) after two crystallisations from chloroform-methanol separated as colourless, felted needles m.p. 165° which gave a yellow tetranitro--methane colour and a pink Liebermann-Burchard reaction.

Found: C, 81.6; H, 11.5.

Calc. for $C_{32}H_{52}O_2$: C, 82.1; H, 11.1%.

$[\alpha]_D$ +15.9° (c, 1.13 in chloroform).

By means of mixed m.p. determinations using a small sample of acetate prepared from a pure specimen of compound E it was ascertained that the acetate m.p. 205° corresponds to E, the m.p. of the acetate m.p. 165° being strongly depressed.

Compound F: Identification as lupeol benzoate.

Compound F gave a yellow tetranitromethane and a pink Liebermann-Burchard colour and crystallised from benzene-acetone as gleaming, transparent, elongated plates m.p. 265° undepressed when mixed with authentic lupeol benzoate (m.p. 265°).

Found: C, 83.6; H, 10.3.

Calc. for $C_{37}H_{54}O_2$: C, 83.7; H, 10.3%.

$[\alpha]_D$ +60.4° (c, 2.03 in chloroform).

lit. value (24) $[\alpha]_D$ +60.4°.

The benzoate was hydrolysed by refluxing for 5 hours with 4% ethanolic potassium hydroxide, the product isolated in the usual manner and acetylated using pyridine and acetic anhydride (1½ hours at 100°). The resulting acetate crystallised from chloroform-methanol as fine needles m.p. 214 - 215° undepressed when mixed

with authentic lupeol acetate (m.p. 215°).

$[\alpha]_D$ +46° (c, 0.83 in chloroform).

lit. value (24) $[\alpha]_D$ +47.5°

Compound G: This substance crystallised from acetone as poorly defined, soft, opaque crystals m.p. 65 - 69° which gave no tetranitromethane or Liebermann-Burchard colourations.

Found: C, 81.5; H, 14.9%.

Fraction 6: Isolation of Compound H.- The yellow-brown oily wax (8g.) on recrystallisation from methanol yielded a colourless, opaque solid (2.8g.) as nodules m.p. 50 - 56°. On slow cooling of a methanolic solution of this solid, a crop (0.36g.) of microneedles separated and after five crystallisations from methanol afforded compound H as very fine needles or prisms m.p. 71 - 73°. The substance gave no colouration with tetranitromethane, no Liebermann-Burchard colour and was optically inactive.

Found: C, 81.4; H, 14.2.

Calc. for $C_{24}H_{50}O$: C, 81.4; H, 14.1.

$C_{26}H_{54}O$: C, 81.7; H, 14.1%.

Isolation of Compound I.- The foregoing methanolic solution of the substance m.p. 50 - 56°, on further cooling, deposited a second crop (0.75g.) of material which was recrystallised thrice from methanol to give compound I as soft, pearly spheres of fine needles m.p. 56 - 57°.

Found: C, 81.5; H, 14.4.
C, 81.4; H, 14.2.
Calc. for $C_{24}H_{50}O$: C, 81.4; H, 14.1.
$C_{26}H_{54}O$: C, 81.7; H, 14.1%.

The compound was optically inactive, gave a very pale yellow tetranitromethane colour, a beautiful royal blue Liebermann-Burchard reaction and showed no selective absorbtion in the ultra-violet.

As a test of homogeneity, the substance was dissolved in benzene-light petroleum and chromatographed on alumina. No fractions differing substantially in m.p. were obtained.

On treatment, in pyridine solution, with 3:5-dinitrobenzoyl chloride (1⅓ hours at 100°) compound I furnished a dinitrobenzoate which separated from methanol as cream-coloured nodules m.p. 73 - 74°. Agreeing analyses onthis compound have not been obtained.

Isolation of β-Amyrin.- The methanolic mother liquor from the original crystallisation of fraction 6, after removal of a further crop (0.66g.) of compound I, was concentrated to small bulk. On cooling the concentrate, a crop (90 mg.) of fine needles m.p. 166° was obtained which on acetylation (pyridine and acetic anhydride) and crystallisation of the acetate from chloroform--methanol gave β-amyrin acetate as needles m.p. 231° undepressed when mixed with an authentic specimen.

Fractions 7 and 8: Isolation of β-Sitosterol (J).- The combined fractions (4.1g.) were recrystallised thrice from methanol from which β-sitosterol (compound J) separated as colourless, lustrous, elongated plates m.p. 137° undepressed when mixed with the sample of β-sitosterol isolated from broom wax (p. 63).

$[\alpha]_D$ -33.4° (c, 1.77 in chloroform).

lit. value $[\alpha]_D$ -34.4°; -34.0°; -34.2° (cf. Table 3)

The colour sequence in the Liebermann-Burchard test was pink → purple → dark blue → green.

Fractions 9 and 10: These fractions were not examined.

Fraction 11: Isolation of Compound K.- The brown, viscous liquid (1g.) was dissolved in boiling methanol, the solution filtered hot and the filtrate allowed to cool. A colourless solid separated (0.13g.) which after one crystallisation from chloroform and seven from benzene yielded compound K as opaque microprisms m.p. 102 - 104.5° which gave no tetranitromethane or Liebermann-Burchard colourations.

Found: C, 78.7; H, 13.7.

C, 78.2; H, 13.8.

Calc. for $C_{26}H_{54}O_2$: C, 78.3; H, 13.6%.

An attempt to prepare an acetyl derivative by heating compound K with acetic anhydride at 100° was unsuccessful.

Fraction 12: Isolation of Compound L.- The oily solid (1.6g.) was recrystallised once from methanol and five times from benzene to give compound L as soft, gleaming plates m.p. 98 - 99°.

Found: C, 75.5; H, 13.2. Active H: 0.60%.

Calc. for $C_{18}H_{38}O_2$: C, 75.5; H, 13.4%. Active H: 0.70%.

Compound L gave no tetranitromethane colour, no Liebermann-Burchard test and showed no selective absorbtion in the ultra-violet.

Identification of Compound L as Octadecane-1:18-diol.- (a) By mixed m.p. comparison. The m.p. of compound L was undepressed on admixture with authentic octadecane--1:18-diol (m.p. 99°).

(b) As the diacetate. A mixture of compound L (70 mg.) and acetic anhydride (2 c.c.) was heated for 2 hours at 100°, the clear solution poured into water and the resulting precipitate extracted with ether. The extract was washed successively with water, sodium hydrogen carbonate and again with water. Evaporation of the dried (sodium sulphate) extract provided a colourless solid which was crystallised thrice from methanol to give the acetyl derivative as small opaque needles m.p. 59 - 60°, undepressed when mixed with octadecane--1:18-diol diacetate (m.p. 60°).

Found: C, 71.5; H, 11.5.

Calc. for $C_{22}H_{42}O_4$: C, 71.3; H, 11.4%.

(c) As the 3:5-dinitrobenzoate. A mixture of compound L (50 mg.), 3:5-dinitrobenzoyl chloride (90 mg.) and pyridine (1 c.c.) was heated for 1 hour at 100°

the pale yellow solution cooled, diluted with ether (50 c.c.) and the ether solution washed successively with 3N hydrochloric acid, 5% sodium hydrogen carbonate and water. Evaporation of the dried (sodium sulphate) solution furnished a yellow-white solid which was recrystallised twice from ethanol, once from acetone and finally from petroleum (b.p. 100 - 120°) to give the di-3:5-dinitrobenzoate as pale yellow microplates m.p. 95° alone and when mixed with a specimen of octadecane-1:18-diol di-3:5-dinitrobenzoate prepared by the above method.

Found: C, 57.8; H, 6.0; N, 7.8.

$C_{32}H_{42}O_{12}N_4$ requires C, 56.9; H, 6.3; N, 8.3%.

(d) As the dibenzoate. A mixture of compound L (100 mg.), pyridine (1 c.c.) and benzoyl chloride (110 mg.) was heated for 1½ hours at 100°. The pale brown solution was then diluted with ether (40 c.c.) and the solution washed successively with dilute hydrochloric acid, dilute sodium hydroxide (2%) and a large volume of water. Evaporation of the dried (sodium sulphate) solution gave a slightly discoloured solid which crystallised from methanol as small, colourless needles m.p. 51° undepressed when mixed with an authentic specimen of octadecane-1:18-diol dibenzoate (m.p. 51°).

(e) By oxidation to octadecane-1:18-dioic acid. To a warm solution of compound L (75 mg.) in stabilised glacial acetic acid (2.5 c.c.) was added dropwise a solution of chromium trioxide (200 mg.) in glacial acetic acid (3 c.c.) and the mixture allowed to stand at room temperature for 30 minutes. It was then poured into water (100 c.c.), the suspension extracted with ether (4 x 25 c.c.), the extract washed repeatedly with water, dried (sodium sulphate) and evaporated to dryness to give an off-white solid (40 mg.) which was treated with warm 2% sodium carbonate, the cloudy solution filtered and the filtrate extracted with ether (2 x 25 c.c.). Evaporation of the ether gave a negligible residue. The sodium carbonate solution was now acidified, the precipitate extracted with ether (3 x 25 c.c.) and the extract washed thoroughly with water. Evaporation of the dried (sodium sulphate) extract provided a colourless solid (30 mg.) which after three crystallisations from chloroform gave the di-acid as small opaque prisms m.p. 124 - 125° alone and when mixed with a sample of octadecane-1:18-dioic acid (m.p. 125°) prepared by hydrolysis of diethyl octadecane-1:18-dioate.

Found: C, 68.2; H, 11.1.

Calc. for $C_{18}H_{34}O_4$: C, 68.8; H, 10.9%.

Diethyl Octadecane-1:18-dioate.- A solution of ethyl hydrogen sebacate (43g.)(25) in absolute methanol (100 c.c.) containing sodium (0.1g.) was electrolysed with a current of 1½ amps (230 volts) between two platinum foil electrodes (4 x 2.5 cm.) set 2 - 3 mm. apart. A vigorous evolution of carbon dioxide occurred and the temperature was maintained at 40 - 50° by means of an acetone/solid carbon dioxide bath for 4 hours when the reaction solution was no longer acid to litmus. On cooling to room temperature the solution set completely solid. The whole was transferred (heat) to a distillation flask, the methanol removed under slight vacuum, the residue dissolved in ether (350 c.c.) and the ether solution washed with 5% sodium hydrogen carbonate (2 x 50 c.c.) and dried (sodium sulphate). Evaporation of the ether gave a colourless, low-melting solid which was recrystallised from methanol to give diethyl octadecane-1:18-dioate (20.7g.; 60% theory) as colourless plates m.p. 48° (lit. value (26):48°).

Octadecane-1:18-diol.- To a solution of lithium aluminium hydride (1.5g.) in dry ether (200 c.c.) was added gradually and with stirring a solution of diethyl octadecane-1:18-dioate (10g.) in dry ether (150 c.c.) at such a rate as to maintain a gentle reflux. When all the ester had been added the mixture was stirred for another 30 minutes and the excess lithium aluminium hydride then destroyed by the gradual addition of water (30 c.c.). The mixture was now diluted with water (100 c.c.), acidified with 10% sulphuric acid (100 c.c.) and a further quantity (200 c.c.) of ether added. After vigorous shaking, the ether layer was separated and the aqueous layer again extracted with ether (2 x 100 c.c.). The combined ether extracts were washed successively with water, dilute sodium hydrogen carbonate and water. Evaporation of the solvent from the dried (sodium sulphate) extracts gave a colourless solid (2.7g.) which after several crystallisations from benzene yielded octadecane-1:18-diol as gleaming plates m.p. 97°. During the extraction of the diol from the reaction mixture considerable trouble had been caused by suspended solid matter. This was now collected, washed with water, dried in vacuo (4.1g.) and recrystallised

twice from benzene to give a further quantity (3.1g., m.p. 98 - 99°) of the diol. The material isolated in this way was found to be much easier to purify than that isolated by ether extraction. The total crude yield (6.8g.) represents 88% of the theoretical.

Table 6 gives a summary of the compounds isolated from the non-saponifiable fraction of the absolute.

Table 6.

Compound.	M.p.	$[\alpha]_D$	Formula.	Character.
A	97°	-	$C_{18}H_{38}O_2$	Octadecane-1:18-diol.
B	66°	-	$C_{20}H_{42}O$ $C_{22}H_{46}O$	Probably long-chain secondary alcohol.
C	235°	+96.6°	$C_{37}H_{54}O_2$	β-Amyrin benzoate.
D	198°	+91.8°	$C_{37}H_{54}O_2$	α-Amyrin benzoate.
E	200°	+35.4°	$C_{37}H_{54}O_2$	Unidentified triterp--ene.
F	265°	+60.4°	$C_{37}H_{54}O_2$	Lupeol benzoate.
G	69°	-	-	Unidentified.
H	73°	-	$C_{24}H_{50}O$ $C_{26}H_{54}O$	Probably long-chain alcohol.
I	57°	-	$C_{24}H_{50}O$ $C_{26}H_{54}O$	Unidentified.
J	137°	-33.4°	$C_{29}H_{50}O$	β-Sitosterol.
K	105°	-	$C_{26}H_{54}O_2$	Probably long-chain diol.
L	99°	-	$C_{18}H_{38}O_2$	Octadecane-1:18-diol.

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